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THE ASSAY, CHARACTERISTICS, COMPOSITION, AND ORIGIN OF OPIUM

No. 8

Codeine and Codeine-Porphyroxine Charts in Relation to the Origin of Opium

In continuation of the international programme of scientific research undertaken pursuant to Resolutions 159 (VII) II C and 246 F (IX), adopted by the Economic and Social Council on 3 August 1948 and 6 July 1949, the Secretary-General is now publishing the present paper, which has been prepared by the Secretariat chemists. This paper gives data on the codeine content of the United Nations samples, and presents and explains charts showing the distribution of codeine values among different producing areas, and the relation between correlated codeine and porphyroxine values and the origin of the samples of opium.

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CODEINE AND CODEINE-PORPHYROXINE CHARTS IN RELATION TO THE ORIGIN OF OPIUM

I. PURPOSE OF THE PAPER

1. The purpose of the present paper is to show the relation of codeine percentages, and of codeine percentages and "porphyroxine-meconidine" values when considered together, to the geographical origin of the opium samples.

2. The new codeine values, obtained on the United Nations opium samples, are first presented and discussed briefly. Then charts are given, based on all the codeine percentages and porphyroxine values obtained by the Secretariat. (Earlier codeine analyses were reported in E/CN.7/202 and porphyroxine values in E/CN.7/195 and ST/SOA/SER.K/4.)

3. The purpose is further to show clearly the great value of classifying a sample of seized opium by its codeine and porphyroxine values, correlated or considered together, as a means of determining the origin of the opium.

II. METHOD OF CODEINE ANALYSIS

4. The method used for codeine analysis was that given in document E/CN.7/202, paragraphs 7 to 13. Any reasonably accurate method for codeine could be used for charts similar to those hereafter presented, but there would have to be some adjustment of the dividing points or boundary lines if the method is changed.

III. CODEINE PERCENTAGES OF THE UNITED NATIONS SAMPLES

TABLE 1.

	China	
U.N. 37 - Received from Taiwan -	lump A	4.12
	lump B	5.15
	lump C	5.10
	lump D	4.53
	Greece	
U.N. 25 -	small loaf, a	1.25
	small loaf, b	1.04
	small loaf, c	1.23
	small loaf, e	1.26
	India	
U.N. 34 - Banaras opium		3.11
U.N. 35 - Malwa opium		3.23
U.N. 36 - Excise opium		3.03
Old Seizure of Standardized opium (redetermination)		3.02
	Iran	
U.N. 18 - Export opium		3.35
U.N. 19 - Export opium		3.22
U.N. 20 - Export opium		3.46
U.N. 21 - Export opium		3.37

/Iran (continued)

	Iran (continued)	
U.N. 47 - Fars		3.71
U.N. 48 - Isfahan		2.46
U.N. 49 - Kerman		3.14
U.N. 50 - Kermanshah		3.35
U.N. 51 - Khorassan		2.93
U.N. 52 - Nehavand		3.35
U.N. 45 - Seizure in France:		
(a) light coloured sticks (porphyroxine - 1.0)		3.39
(b) an average dark stick (porphyroxine - 8.0)		2.64
U.N. 53 - Seizure (porphyroxine - 6.2)		3.45
	Korea	
U.N. 16 - Korea No. 1 - A		1.31
No. 1 - B		1.23
(Korea No. 1 is substandard or adulterated. Lump A assayed only 4.36 percent of morphine)		
U.N. 17 - Korea No. 2		2.45
52844 - From Korea factory at close of World War (1945). (porphyroxine - 9.6)		4.22
	Laos	
U.N. 26 - Laos, Luang Prabang,	lump A	2.14
	lump B	1.93
	lump C	1.77
U.N. 27 - Laos, Xieng Khouang,	lump A	1.79
	lump B	2.41
	Turkey	
U.N. 1 - Turkey No. 1 - Aydin -	lump A	1.32
	lump B	2.12
	lump C	2.33
	lump D	1.96
	lump E	1.46
	lump F	1.61
	lump G	1.50
	lump H	1.64
U.N. 2 - Turkey No. 2 - Afyon -	lump A	2.19
	lump B	2.14
	lump C	1.67
U.N. 3 - Turkey No. 3 - Çorum -	lump A	1.21
	lump B	1.35
	lump C	2.02
	lump D	1.00
U.N. 4 - Turkey No. 4 - Çal -	lump A	1.55
	lump B	0.90
	lump C	1.20
	lump D	2.36

/Turkey (continued)

Turkey (continued)

U.N. 5 - Turkey No. 5 - Usak -	lump A	1.28
	lump B	0.78
	lump C	0.90
	lump D	0.97
U.N. 6 - Turkey No. 6 - Nallihan-	lump A	0.80
	lump B	0.92
	lump C	1.12
	lump D	1.17
	lump E	1.27
U.N. 7 - Turkey No. 7 -Merzifon-	lump A	1.45
	lump B	1.67
	lump C	1.04
	lump D	1.77
U.N. 8 - Turkey No. 8 - Burdur-	lump A	1.85
	lump B	0.94
	lump C	0.85
U.N. 9 - Turkey No. 9 - Kula -	lump A	2.32
	lump B	1.55
	lump C	1.62
	lump D	1.45
U.N. 10 - Turkey No. 10 - Sandikli -	lump A	0.84
	lump B	0.97
	lump C	1.42
U.N. 11 - Turkey No. 11 - Aksehir -	lump A	1.00
	lump B	1.77
	lump C	1.17
U.N. 12 - Turkey No. 12 - Zile -	lump A	1.56
	lump B	2.25
	lump C	3.81
	lump D	3.67
	lump E	1.65
	lump F	2.41

(Zile lump A is adulterated with cornstarch; nevertheless, it assayed 11.1% percent in morphine.)

U.N. 13 - Turkey No. 13 - Isparta -	lump A	1.37
	lump B	1.78
	lump C	0.75
U.N. 14 - Turkey No. 14 - Malatya -	lump A	3.04
	lump B	2.38
	lump C	2.99
	lump D	2.11
	lump E	2.85
U.N. 15 - Turkey No. 15 - Export opium		1.08
U.N. 29 - Exhibition - A, half loaf		2.52
B, whole loaf		2.16
U.N. 30 - Exhibition, standardized opium		0.94
U.N. 31 - Exhibition, reduced size cubes		1.15
U.N. 40 - Purchased at Eskiser		1.65

/Turkey (continued)

	Turkey (continued)	
U.N. 41 -	Purchased at Kutahya - ball, A	0.35
	lump, B	1.97
	(Sample A is greatly substandard or adulterated, and assayed only 4.2 percent morphine. In ST/SOA/SER.K/4 the porphyroxine values for U.N. 41A and U.N. 42 were interchanged in error.)	
U.N. 42 -	Purchased at Afyon	1.50
U.N. 44 -	Loaf in copper vessel	1.97
U.N. 46 -	Seizure in France (porphyroxine 4.2)	1.92
	Union of Soviet Socialist Republics	
U.N. 39 -	Sample received from France	1.75
	Vietnam	
U.N. 28 -	Tonkin, Laokay, lump a	1.89
	lump b	1.87
	lump c	2.12
	lump h	2.86
	lump j	2.75
	lump m	3.16
	Yugoslavia	
U.N. 24 -	Yugoslavia No. 1 - Export type	1.16
U.N. 38 -	Yugoslavia No. 2 - small loaf, a	1.19
	small loaf, c	1.46
	small loaf, d	1.20
	small loaf, h	1.48

IV. DISCUSSION OF THE NEW CODEINE DATA

5. In general, the results followed the same pattern found in the previous study (E/CN.7/202). The highest new codeine value found for either Greece or Yugoslavia was 1.46 percent, whereas the lowest new value found for either Iran or India was 2.46 percent, nearly all the new values for Iranian opium and all three new values for Indian opium being, in fact, above 3 percent. The samples of Chinese opium were extremely high in codeine, above 4 percent and two even above 5 percent. This is in accord with the previous finding that samples from the Far East, at any rate from China, Korea, and Mongolia, are the highest of all in codeine.

6. Most Turkish opium is in the low-codeine class, like that of Yugoslavia and Greece. However, some high-codeine opium is produced in Turkey, especially in Malatya and Zile. In the rest of Turkey only a minority of the samples from any one province or district exceed 1.9 percent in codeine (ranging up to 2.65 percent), and these samples moderately high in codeine are not confined to districts producing "soft" opium, but occur also in the "druggist" areas.

/7. Values

7. Values quite close to 2 percent, which were almost missing from the preceding tabulation, are now supplied to some extent by opiums from Indochina (Laos and Vietnam) as well as from Turkey. (See Charts 2 and 3 for the distribution of the values, based on all the data.)

V. REPRESENTATION OF THE CODEINE PERCENTAGES AND PORPHYROXINE VALUES

8. All the charts have the codeine values on the same scale. In all except Chart No. 2 the codeine percentages are used as ordinates; in Chart No. 2 they appear as abscissae. Charts 1 to 3 concern only codeine; Charts 4 to 11 concern both codeine and porphyroxine.

9. On Charts 4 to 11, the Lovibond values of the red colour obtained from the alkaloid "porphyroxine-meconidine" are represented as abscissae. These values are called the porphyroxine values, for short, in this paper. Since most of the samples have porphyroxine values below 30, those showing higher and higher values becoming fewer and fewer, the scale is gradually compressed above this point, at the end the space for values from 50 to 80 being no more than one-fourth that from 0 to 30. The same porphyroxine scale is used on all the Charts 4 to 11.

10. Values previously published are used in all cases with the exception of one redetermination, the codeine percentage of the seizure sample of standardized Indian opium (originally analyzed several years ago, before the method was well established) being raised from 2.25 to 3.02 percent. Samples known to be greatly substandard are omitted from the charts. The few such samples omitted all assay less than 6 percent morphine. Such samples can only be dealt with by means of ratios, not by absolute percentages of the alkaloids. Studies of the ratios will be given later in order that seizures of adulterated and substandard samples may be dealt with. The Zile sample adulterated with cornstarch (U.N. 12A, Turkey) is omitted from the charts distinguishing the Malatya and Zile samples from those of the rest of Turkey, although it is still above 10 percent in morphine.

VI. DISCUSSION OF THE CODEINE CHARTS

Chart No. 1

11. Chart No. 1 shows the curves of codeine percentages in the same way that "porphyroxine-meconidine" values were shown in document ST/SOA/SER.K/4. The codeine percentaces are used as ordinates. Horizontally, the samples for each producing area are in order of the percentage and are spaced equally apart on the curve for that area. The spacing varies for the different areas, being greater for a lesser number of samples.

12. The curve for Afghanistan is not represented chiefly because it would virtually coincide with the curve for India. All the Turkish samples are represented on one curve, but the five highest values (above the mark across the curve) are all from Malatya and Zile. In later charts the samples from Malatya and Zile are

/separated

separated or distinguished from those for the rest of Turkey. The position of the export samples on the curve for Turkey - all on the lower half of the curve - indicates that the high-codeine samples are over-represented in the United Nations samples, if the quantity of production is considered.

13. Although Chart No. 1 does not give the most useful representation of the results for the purpose of determining the origin of seizures, it does bring out certain points, such as the generally slightly higher trend of samples from Iran as compared with those from India. It may also be noted that the lower half of the curve for Turkey will very nearly coincide with the curve for Yugoslavia and Greece (if it be moved over); and this characteristic of the bulk of Turkish production, as shown by the position of the export samples on the Turkish curve.

14. The greatest gap between the median values is between the curve for Laos and Vietnam and the curve for India. A line at 2.4 percent will divide the curves rather well into two main families, with little overlapping, except for the intermediate character of the curve for Laos and Vietnam.

Chart No. 2

15. The style of representation in Chart No. 2 is the same as that used for the chart in document E/NC.7/202. The codeine values are the abscissae, but are taken only to the nearest 0.1 percent; the ordinates for each curve represent the number of samples found at that percentage.

16. The groups are more obvious on the chart than if explained in words. The effect of dividing lines at 1.6, 2.0, and 2.7 percent may be noted.

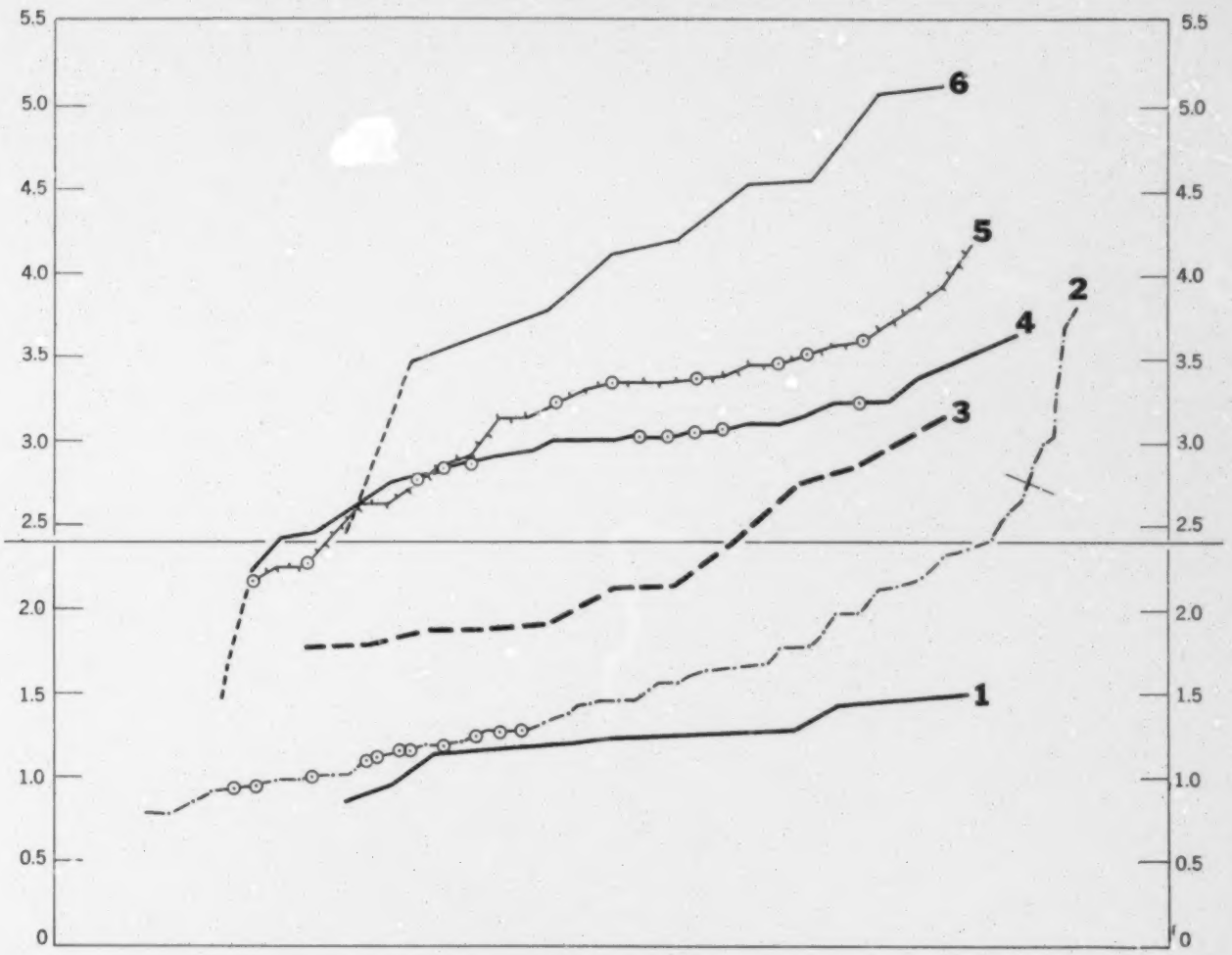
Chart No. 3

17. In Chart No. 3 the codeine values are ordinates and are entered at the exact value analytically determined, as close as can be, without overlapping of the spots, to the vertical line for each area.

18. This is the most useful way of representing the codeine values for the purpose of noting the probable origin of an opium seizure as indicated by its codeine percentage. Boundary lines may be taken for greatest convenience and with sufficient accuracy at 0.5, 1.33, 2.0, 3.0, 3.5, 4.0, 4.5, and 5.5 percent as indicated on the chart.

CHART NO. 1

CURVES OF CODEINE PERCENTAGES FOR SIX OPIUM-PRODUCING REGIONS



KEY: 1. Greece and Yugoslavia (16 samples)
2. Turkey (85 samples)
3. Laos and Vietnam (11 samples)
4. India (29 samples)
5. Iran (27 samples)
6. China, Korea, Mongolia (10 samples)

Ordinates are the percentages of codeine.

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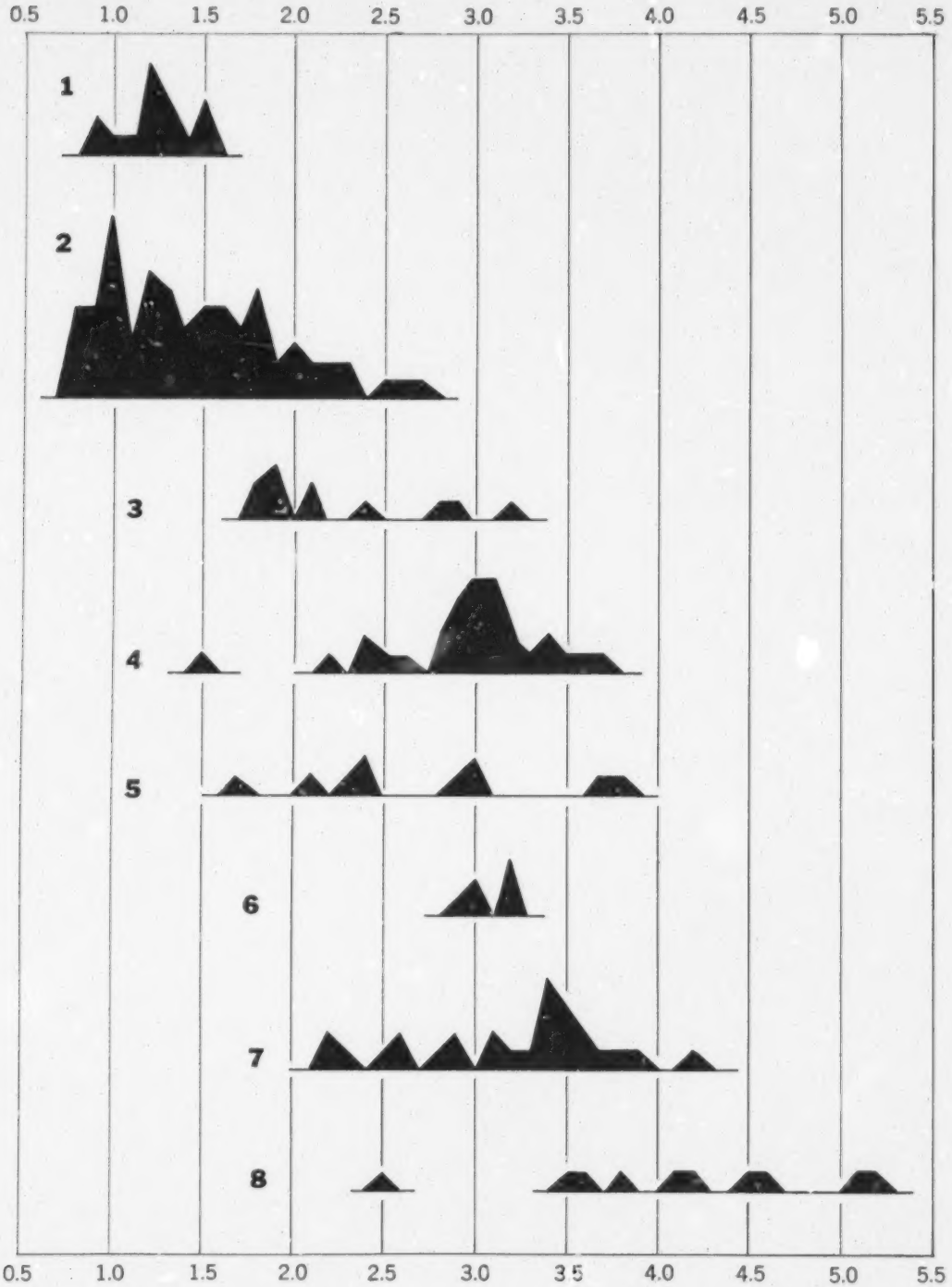
CHART NO. 2

DISTRIBUTION OF THE CODEINE GROUPS FOR EIGHT OPIUM-PRODUCING REGIONS



KEY:
1. Greece and Yugoslavia
2. Turkey, excluding Malatya and Zile
3. Laos and Vietnam
4. India
5. Turkey, Malatya and Zile only
6. Afghanistan
7. Iran
8. China, Korea, Mongolia

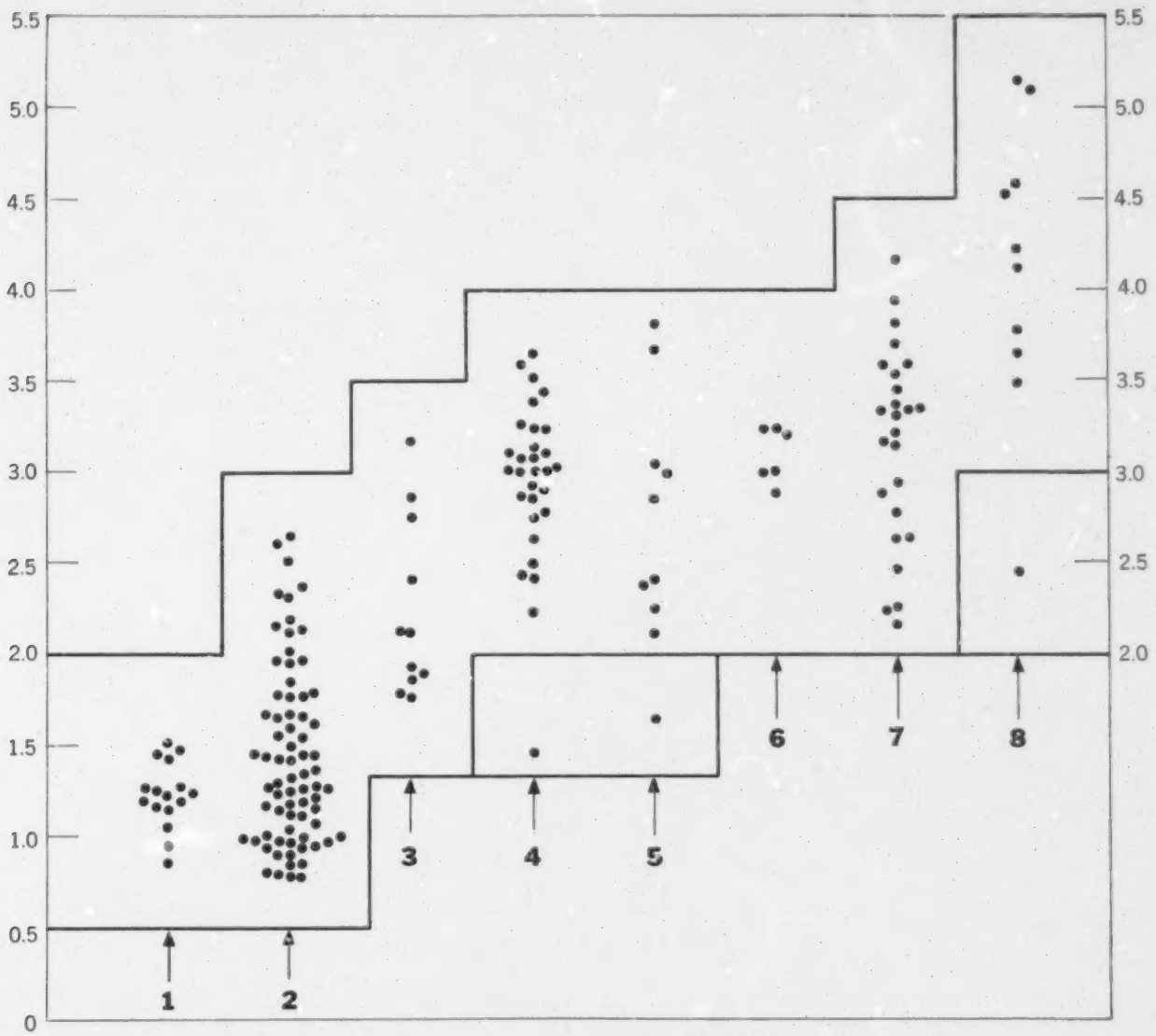
Abscissal are the codeine percentages (taken to the nearest tenth).

Ordinates for each curve separately are the number of samples at the particular percentage.

UN PRESENTATION 2408.2

CHART NO. 3

DISTRIBUTION OF THE CODEINE PERCENTAGES OF OPIUM FOR EIGHT PRODUCING REGIONS



KEY:
1. Greece and Yugoslavia
2. Turkey, excluding Malatya and Zile
3. Laos and Vietnam
4. India
5. Turkey, Malatya and Zile only
6. Afghanistan
7. Iran
8. China, Korea, Mongolia

Ordinates are the codeine percentages.

For each sample a spot is placed at the correct height for the codeine percentage according to the laboratory determination, and as close as possible, without overlapping, to the vertical line for the particular region.

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KEY FOR CHARTS N° 4 TO N° 11

KEY:

AFGHANISTAN

△ CHINA, KOREA and MONGOLIA

○ GREECE

\+ INDIA

● IRAN

▽ LAOS and VIETNAM

○ TURKEY excluding MALATYA and ZILE

⊗ TURKEY, MALATYA and ZILE only

▲ YUGOSLAVIA

Ordinates: Codeine percentages

Abscissal: Porphyroxine values

KEY FOR CHARTS N° 4 TO N° 11

KEY:

AFGHANISTAN

△ CHINA, KOREA and MONGOLIA

○ GREECE

\+ INDIA

● IRAN

▽ LAOS and VIETNAM

○ TURKEY excluding MALATYA and ZILE

⊗ TURKEY, MALATYA and ZILE only

▲ YUGOSLAVIA

Ordinates: Codeine percentages

Abscissal: Porphyroxine values

VII. DISCUSSION OF CHARTS 4 TO 10

Chart No. 4

19. Chart No. 4 shows the position of the export samples of India, Iran, Turkey, and Yugoslavia, plotted for codeine percentage and porhyroxine value. Three quite distinct areas are indicated, the export samples of Turkey and Yugoslavia occupying virtually the same area.

Charts No. 5, 6, 7 and 8

20. Charts No. 5, 6, 7 and 8 show the codeine-porhyroxine positions for all the samples (except a very few which were found adulterated or greatly substandard) from four main producing regions: Chart No. 5, the Far East (China, Korea, Mongolia); No. 6, India; No. 7, Iran; and No. 8, Turkey. By superimposing one of these charts upon another the extent to which the areas of the chart overlap one another can be easily seen. The overlapping is not very great: the majority of the samples of one region always fall outside the area charted for any one of the other three regions.

Chart No. 8

21. On Chart No. 8 it will be noted that the area characteristic of samples from Malatya and Zile is quite separate from the area for the rest of the Turkish samples. The samples from Malatya and Zile do not form as compact a group as the samples from other regions; still, 7 out of 10 of these samples fall in the characteristic area of fairly high codeine percentage and extremely high porphyroxine value. These samples represent a unique type of opium, which quite possibly may be produced to some extent in some other parts of Turkey, though not otherwise represented among the available Turkish samples. Conversely, some of the "normal" Turkish opium may be produced on some farms in the Malatya and Zile districts.

Charts No. 9 and 10

22. Chart No. 9 shows on one chart the areas for samples from Afghanistan, Indochina (Laos and Vietnam), and Greece and Yugoslavia. (The opium-producing areas for both Greece and Yugoslavia, and Bulgaria as well, are in Macedonia.) These areas can be drawn without overlapping each other, except that one Indochina sample is "out of place". Of course, these areas overlap some of those shown in Charts 5 to 8.

23. Chart No. 10 shows on one chart all the samples shown on Charts 5 to 8. Chart No. 10 shows the very great degree to which the five areas can be drawn without substantial overlapping and with very few samples represented in the wrong area. Of course, the boundaries are somewhat arbitrary, and if more samples were examined the overlapping would probably increase, but the clustering of samples would also increase, doubtless even more conspicuously.

24. If Chart No. 9 is superimposed on Chart No. 10, it will be seen that most of the samples from Afghanistan correspond to the higher-porphyroxine samples from Iran; most of those from Laos and Vietnam correspond to the higher-codeine samples of Turkey (without Malatya and Zile); and the samples from Greece and Yugoslavia correspond to

/the area

the area of greatest clustering of the samples from Turkey. (The Afghan samples may also be compared to the low-porphyroxine samples of India as shown on Chart No. 6.)

VIII. USE OF THE CHARTS WITH SEIZURE SAMPLES

25. If the coordinates (codeine percentage and porphyroxine value) of a sample of seized opium are noted on Chart No. 10, they usually show clearly the geographical region from which the opium probably came, or at least, greatly limit the possibilities. Of course, other regions, such as those represented on Chart No. 9, sometimes have to be taken into consideration also.

26. When the position for a seizure sample does not correspond to an area where the characteristic samples cluster, but rather is on or near a boundary line, or where areas may overlap, and when a decision must be made between such corresponding areas as are mentioned in paragraph 24, it is obvious that one or more characteristics in addition to codeine percentage and porphyroxine value must be used. However, aside from rare instances of very abnormal opium, the codeine-porphyroxine charts always limit the possibilities for any particular sample to a very few regions, and in addition show what kind of samples from those regions should be taken for further comparisons.

27. Chart No. 11 shows the allocation of 15 seizure samples to three different regions of production. Although in the case of most of these samples there was other evidence of the origin, it surely would not be hard to decide the probable origin upon purely chemical grounds.

CHART NO. 4

CODEINE - PORPHYROXINE VALUES FOR SAMPLES OF EXPORT OPIUM FROM INDIA, IRAN, TURKEY AND YUGOSLAVIA

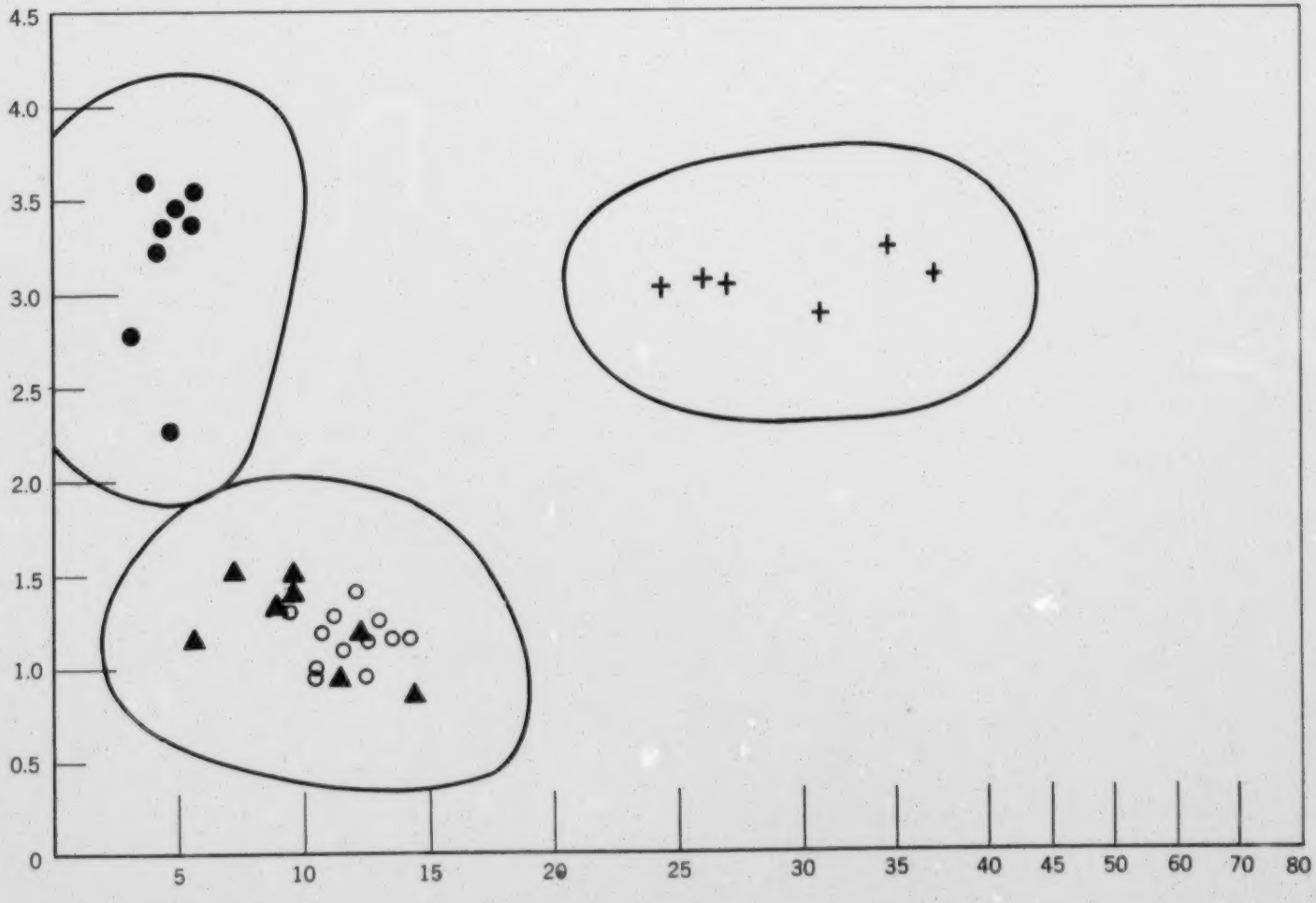

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CHART NO.5

CODEINE - PORPHYROXINE VALUES FOR OPIUM SAMPLES FROM CHINA, KOREA, MONGOLIA

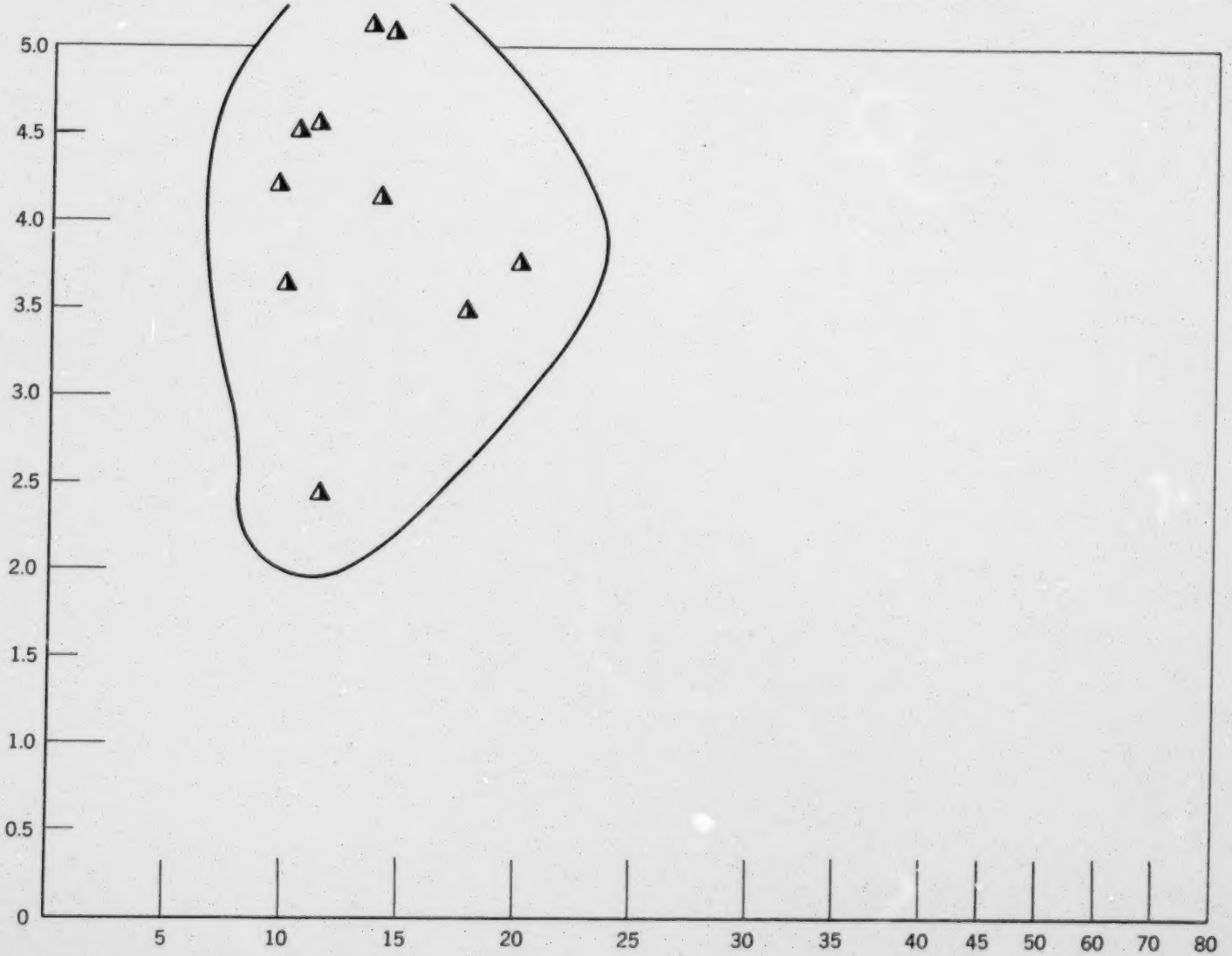

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CHART NO.6

CODEINE - PORPHYROXINE VALUES FOR OPIUM SAMPLES FROM INDIA

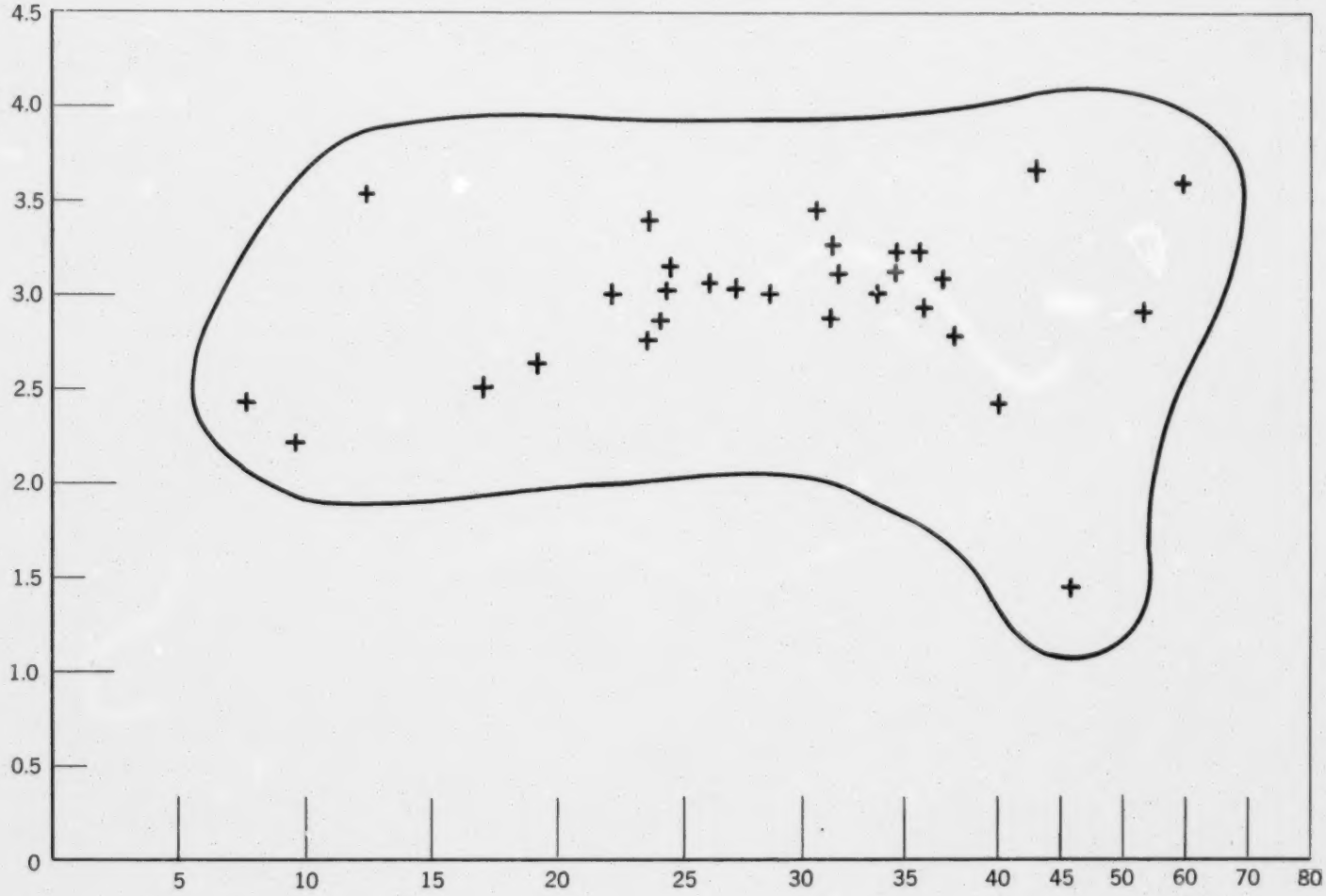

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CHART NO. 7

CODEINE - PORPHYROXINE VALUES FOR OPIUM SAMPLES FROM IRAN

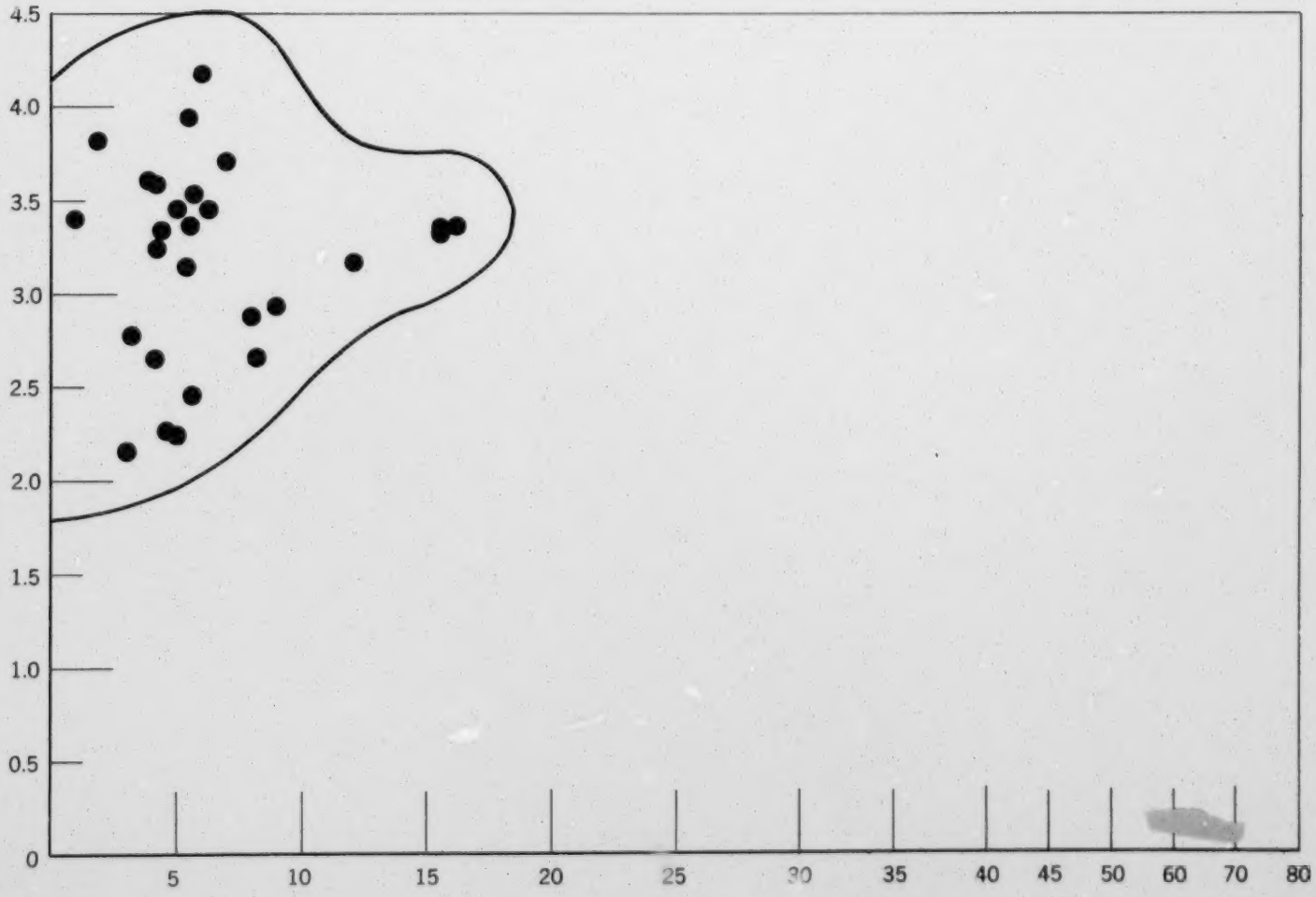

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CHART NO. 8

CODEINE - PORPHYROXINE VALUES FOR OPIUM SAMPLES FROM TURKEY

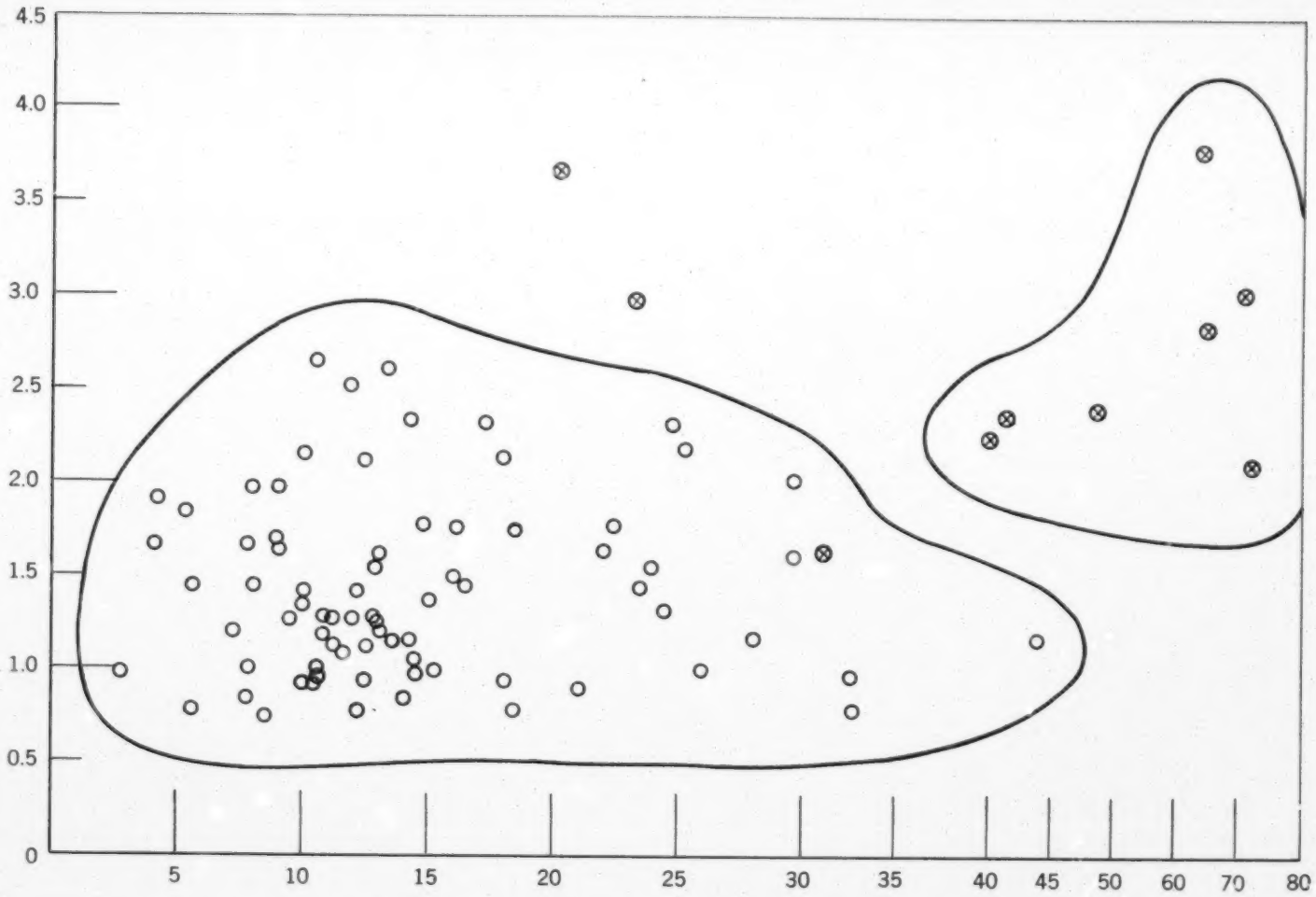

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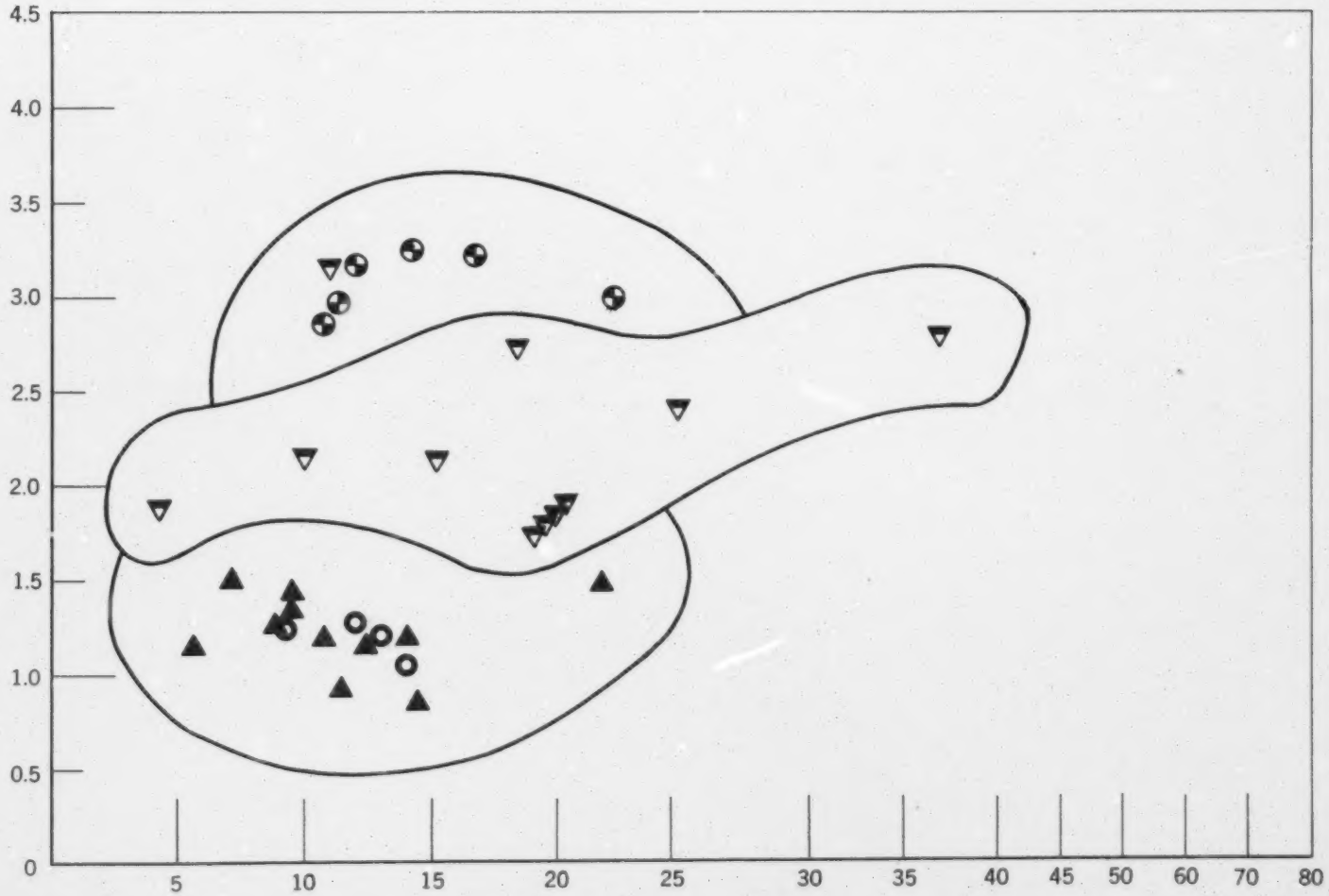
CHART NO. 9

CODEINE - PORPHYROXINE VALUES FOR OPIUM SAMPLES FROM THREE PRODUCING REGIONS: AFGHANISTAN, LAOS AND VIETNAM, YUGOSLAVIA AND GREECE



UN PRESENTATION 2408 .9

CHART NO. 10

CODEINE - PORPHYROXINE VALUES FOR OPIUM SAMPLES FROM FIVE MAIN PRODUCING REGIONS:
CHINA, KOREA, MONGOLIA; INDIA; IRAN; TURKEY, EXCLUDING ZILE AND MALATYA; TURKEY, ZILE AND MALATYA ONLY

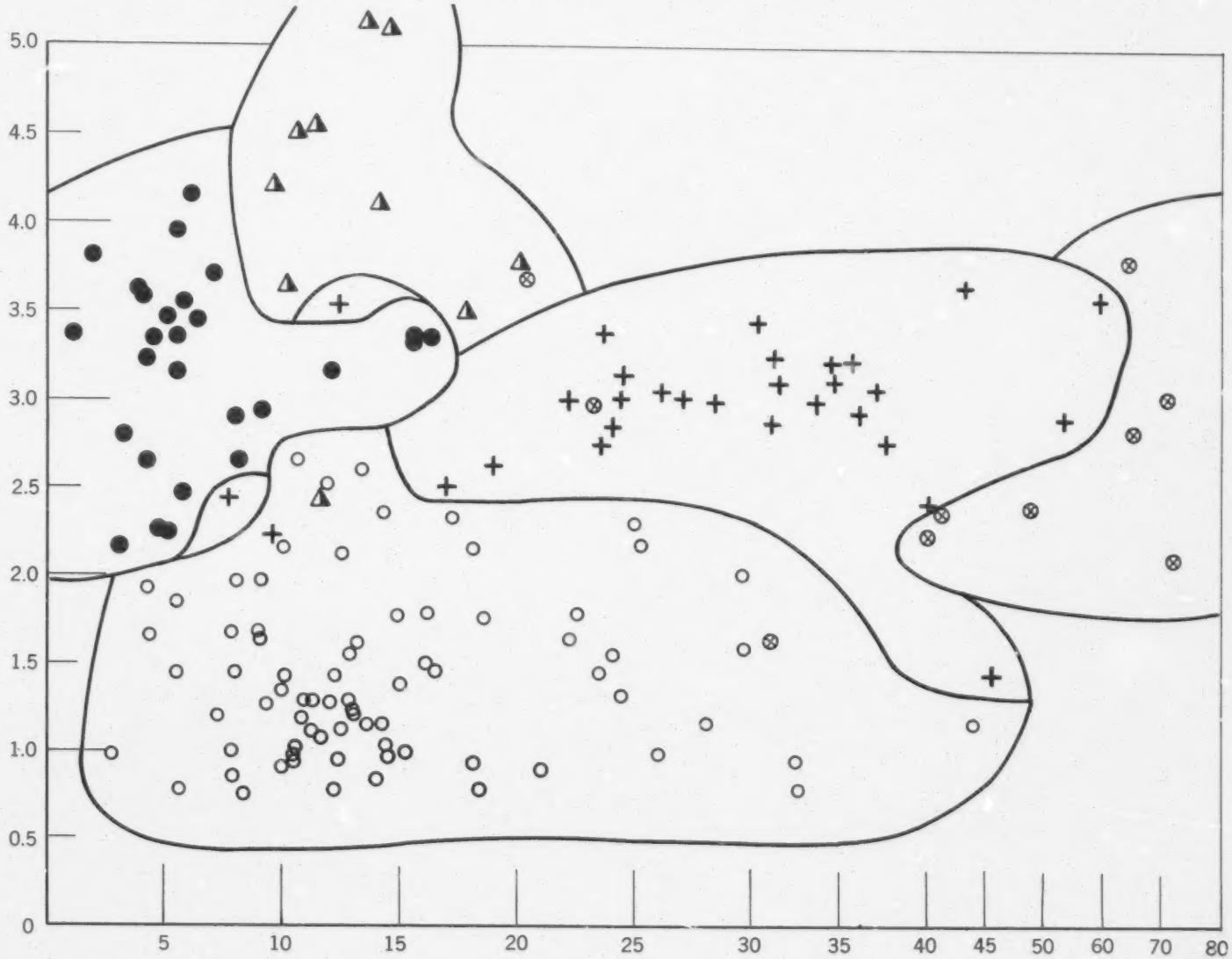


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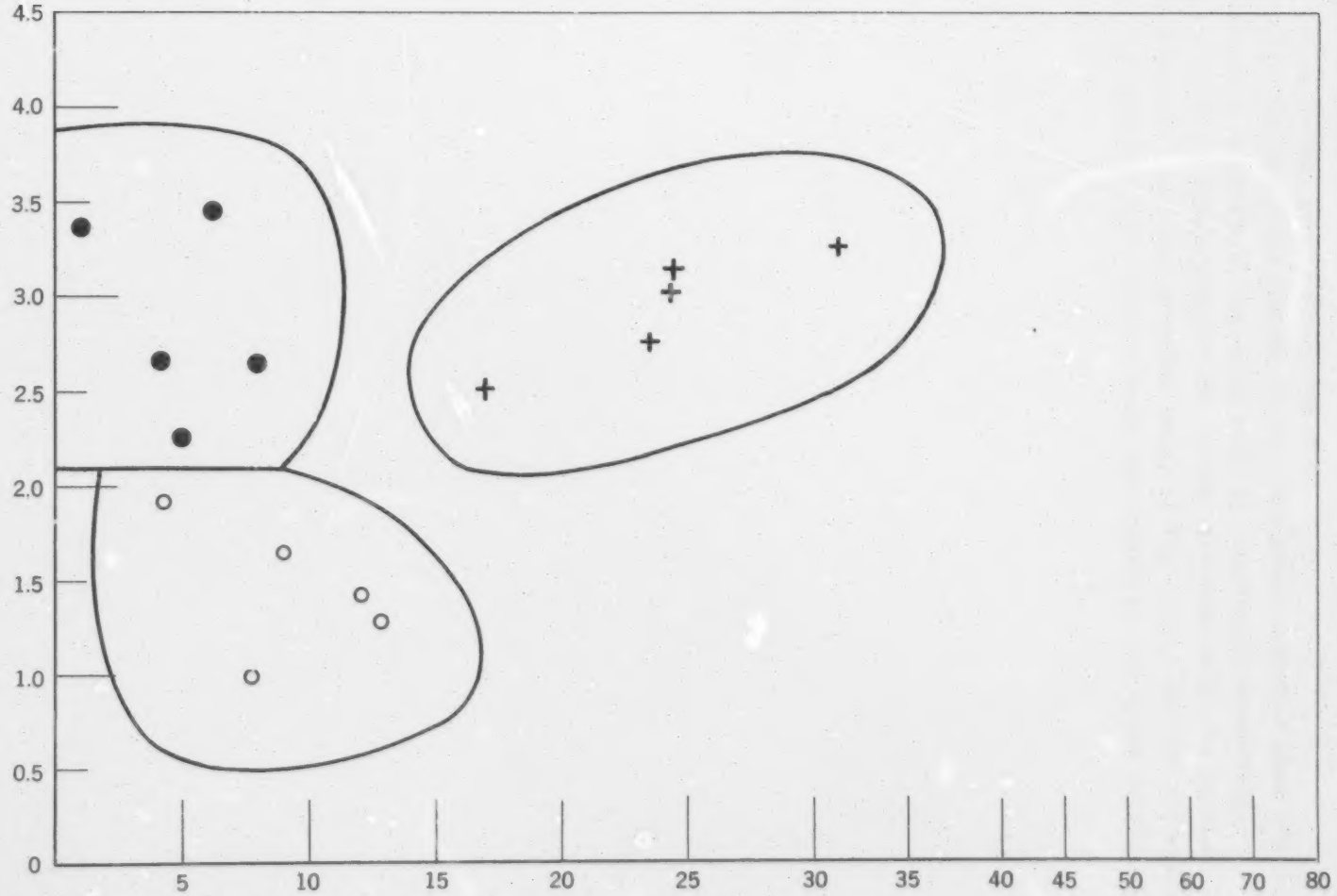
CHART NO. 11

CODEINE - PORPHYROXINE VALUES FOR FIFTEEN SAMPLES OF SEIZURES OF OPIUM



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IX. CONCLUSIONS

28. The codeine values fall in fairly well-defined groups, which in the main correspond to the different geograph cal regions.

29. The codeine and the porphyroxine values considered together show the origin of the opium much more definitely than when each is considered separately.

30. The correlated codeine-porphyroxine values, together with the general appearance, microscopic test, and morphine assay of the opium, and the circumstances attendant upon the seizure, will usually make the origin of the opium fairly obvious.

- - - - -

UNITED NATIONS

SECRETARIAT

ST

GENERAL

ST/SOA/SER.K/9
9 June 1952

ORIGINAL: ENGLISH

THE ASSAY, CHARACTERISTICS, COMPOSITION, AND ORIGIN OF OPIUM

No. 9

Elimination of the other Opium Alkaloids in the Assay of Opium for Morphine

In continuation of the international programme of scientific research undertaken pursuant to resolutions 159 II C (VII) and 246 F (IX), adopted by the Economic and Social Council on 3 August 1948 and 6 July 1949 respectively, the Secretary-General is presenting the following paper prepared by the United Nations Secretariat chemists in which the discussion on the assay of opium for morphine is continued.

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ELIMINATION OF THE OTHER OPIUM ALKALOIDS IN THE ASSAY OF OPIUM FOR MORPHINE

Object of the paper

1. The present paper relates to the method for assaying the morphine content of opium as described in document ST/SOA/SER.K/1.

2. In order to prove that no substantial amount of any other alkaloid can be present in the final morphine residue a thorough study has been made of what becomes, during the morphine extraction process, of all other alkaloids known to be present in opium and of several others which might possibly be present. Many of the observations made during the study are applicable also to other methods of assaying opium for morphine.

The Major Alkaloids of Opium

3. The six principal opium alkaloids other than morphine, all of them easily detected in appropriate extractions even of very little opium, are: narcotine, papaverine, codeine, thebaine, cryptopine, and narceine.

4. Narcotine is scarcely present in appreciable amount in the lime-water extract of opium; any portion that may get into the ether-acetone extract would be very readily extracted by chloroform from dichloracetic acid solution.

5. However, one more possibility for narcotine should be considered. If the lactone ring opens, it becomes soluble in alkali, forming a narcotinate. It may then stay with morphine in extraction methods which depend on the solubility of morphine in alkali, and this possibility has sometimes been overlooked. Moreover, any narcotine in the final residue will show some titration, although it is too weak a base to be accurately titrated in the usual way. In order to study this possibility in the United Nations method, narcotine was heated in a solution of alcoholic KOH and thus converted to potassium narcotinate. The alcoholic solution was diluted with water; the narcotine-acid was then set free by addition of ammonium salt, and extracted with chloroform. The residue, upon evaporation of the solvent, was not readily soluble in lime-water, but a little dissolved. After addition of ammonium chloride and continuous extraction with ether-acetone, a small residue of the narcotine-acid was obtained. However, after solution in dichloracetic acid - whether or not this spontaneously reconverted it to narcotine - it was readily extracted by chloroform. The narcotine therefore cannot get into the final morphine extract.

/6. Papaverine

6. Papaverine is present only in very small amounts in the lime-water solution. Like narcotine, it is very readily extracted by chloroform from dichloracetic acid solution. In fact, both narcotine and papaverine can be shaken out gradually from dichloracetic acid solution even by means of carbon tetrachloride when this solvent is used instead of chloroform.

7. Codeine is the second alkaloid to morphine in the lime-water extract, and of course it is extracted by the ether-acetone. It is the most difficult of the opium alkaloids to separate from morphine by the dichloracetic-acid method. However, an excellent separation is made, although it normally requires shaking with from 8 to 14 separate portions of chloroform, and occasionally even more. Codeine could be more readily separated by extraction from alkali, but as the acid separation would still be necessary to separate the phenolic "alkaloid 3", it was decided to use only the acid separation for the regular method of assay.

8. Thebaine is present in the lime-water solution in much greater amounts than might be expected, considering its insolubility as the free base in an alkaline solution. (In one case, in connexion with a codeine determination made on the lime-water extract, thebaine equivalent to 2% of the opium was found.) Thebaine is extracted by chloroform from dichloracetic acid much more readily than codeine though not quite so readily as papaverine.

9. Cryptopine closely accompanies codeine in extractions. It is extracted more readily than codeine by chloroform from dichloracetic acid solution.

10. Narceine is not a phenol, like morphine, but it has a carboxylic acid group which makes it soluble in alkali. In most methods of determining morphine by extraction, narceine accompanies morphine throughout, though it is so feeble as a base that it may not affect the titration. Its isoelectric point is at about pH^6, while that of morphine is about pH^9. In the United Nations method, the adjustment of the pH for the extraction of morphine, and the insolubility of narceine in ether-acetone, are not conducive to its extractions, and practically all of it that is extracted from the opium by the lime-water, remains in the original aqueous solution. Any portion that does go into the ether-acetone extract is very readily shaken out in Stage III. In fact, narceine is more readily extracted by chloroform from dichloracetic acid solution than from an ammoniacal aqueous suspension of the freshly precipitated base.

/The Minor

The Minor Non-Phenolic, Non-Acidic Alkaloids

11. In addition to the preceding alkaloids there are a number of minor alkaloids in opium, both non-phenolic and phenolic. The non-phenolic (and non-acidic) minor alkaloids are: laudanosine, which is closely related to papaverine, but a stronger base; neopine, an isomer of codeine; protopine (or fumarine), closly related to cryptopine; papaveramine, of unknown structure; xanthaline (papaveraldine), an oxidation product of papaverine; and hydrocotarnine, a hydrolysis product of narcotine. The last two are very probably not natural alkaloids of the poppy plant.

12. Neopine is, like codeine, extracted gradually by chloroform from dichloracetic acid solution.

13. Protopine (fumarine) is extracted fairly readily, like cryptopine (to which it is closely related), by chloroform from dichloracetic acid solution.

14. The remaining known minor non-phenolic alkaloids were not available for individual study; but, from their relationships, it is probable that they are all likewise extracted by chloroform from dichloracetic acid solution. At any rate, they would be extracted by chloroform from alkali solution. This separation is not part of the United Nations method, but it has been applied several times after titration of the morphine, and in no case has any important error been discovered. Generally the alkaloid thus recovered has been less than 0.07% of the opium by titration and even this small amount seemed to consist mainly of residual traces of codeine rather than some minor alkaloid. It is therefore certain that the non-phenolic, non-acidic alkaloids of opium, whether major or minor, do not introduce any substantial error into the morphine determination, either individually or in the aggregate.

The Minor Phenolic Alkaloids and Little-Known Bases

15. The minor phenolic alkaloids of opium may be divided into two classes, those extractable by chloroform from alkali solution, and those not so extractable. In the past this distinction was not recognized: it was assumed that with any organic solvent, the minor phenolic alkaloids must stay with morphine when the non-phenolic alkaloids are separated by shaking out an alkali solution; but on the basis of reported percentages for these minor alkaloids it was thought, or at least hoped, that if there were an error, it would be a slight one. Actually most of the minor phenolic alkaloids are separated from morphine when chloroform is used for the

/alkali

alkali extraction, but even so the positive error caused by a phenolic alkaloid that stays with the morphine is about 0.3% of the opium, while if some less-effective solvent is used, it may perhaps be twice as much. (These positive errors in extraction methods were, however, often balanced or overbalanced by negative errors.)

16. In extractive studies made at the beginning of the present research, the little-known and obscure alkaloids of opium that could be recognized in some way were first distinguished by number as follows: alkaloid 1, the alkaloid that yields a purplish red colour on heating in solution with mineral acid ("porphyroxine-meconidine"); alkaloid 2, the chief phenolic alkaloid extractable by chloroform from alkali solution (laudanine); alkaloid 3, the chief phenolic alkaloid (after morphine) not extractable by chloroform from alkali solution; alkaloid 4, insufficiently studied, but meant to be the chief "alkaloidal" substance not readily extracted from aqueous solution by immiscible organic solvents, but extracted to some extent by the combinations of solvents such as are used to extract morphine, by reason of some solubility in the water-miscible component of the solvent mixture (e.g. isopropanol). These four probably account for all the phenolic or acidic bases, after morphine and narceine, that may amount to or possibly exceed 0.1% of the opium. In extractive separations on a larger scale the following have also been distinguished, all apparently of phenolic character: alkaloid 5 which gives a colour reaction like that of thebaine with sulphuric acid; alkaloid 6, a very weak base and extractable by chloroform from alkali solution; alkaloid 7, which closely accompanies alkaloid 2 (and like it, is extractable by chloroform from alkali solution), but which yields colour reactions like those of alkaloid 3.

17. Alkaloid 1 --- referring to the true alkaloid which has been erroneously described under the names "porphyroxine" and "meconidine" --- is apparently phenolic, or at any rate, soluble in alkali, but is extractable by chloroform from alkali solution, and also from dichloracetic acid solution. Contrary to statements in the literature, there is no "porphyroxine" that is insoluble in alkali, nor is there any "meconidine" that is not extractable by chloroform from alkali. Rhoeadine, another alkaloid which yields a red colour by heating its solution in dilute mineral acid, is not an alkaloid of opium though often so listed.

18. Laudanine has been found, contrary to previous statements, to be readily extracted from alkali solution (2% NaOH) by chloroform. It is also readily

/extracted

extracted by chloroform from dichloracetic acid solution. This was ascertained both on known laudanine and on the "alkaloid 2". The known laudanine was obtained from the Späth collection, through the kindness of Professor Fuchs, Head of the Pharmacognostical Institute of the University of Vienna, Austria. Laudanidine (tritopine) is an optical isomer of laudanine and is doubtless included in the extract of "alkaloid 2" from opium.

19. "Alkaloid 3" is not extractable from alkali solution by chloroform, and accompanies morphine in most extractions. It is, however, separated from morphine by extraction with chloroform from dichloracetic acid solution, six or seven shakeouts being required. It can also be separated from morphine by the far greater solubility of the free base in carbon tetrachloride; but this is difficult to apply to extractions unless the quantities are so small that the morphine does not precipitate during the shakeout with carbon tetrachloride; and the separation is also less complete than with chloroform and dichloracetic acid solution, unless the process is repeated once or twice. It was chiefly to separate this alkaloid that the dichloracetic acid separation was worked out. Probably either this base or "alkaloid 7" is to be identified with codamine. Codamine is said not to be extracted from alkali by chloroform and on this basis it should be "alkaloid 3"; but it seems probable that the same mistake was made as for "meconidine" and laudanine, and that it is "alkaloid 7" that is really codamine.

20. Other solvent-combinations used for morphine take out more extraneous material from opium solutions than does 60:40 ether-acetone. If a continuous extraction is made with ether-acetone as in the United Nations method, until the morphine is all out, and then benzene-isopropanol (3:1) is used, a two or three hour extraction brings over substances amounting to some 2 or 3 per cent of the opium and yielding precipitates with the "general alkaloidal reagents". A substantial part of this material is extracted by chloroform from dichloracetic acid solution, including a substance giving a strong blue or green colour with Fröhdés reagent. The small amount of the remaining material that may be re-extracted by the morphine solvents apparently does not yield any titration, as long as the residue does not contain any traces of residual morphine or "damaged" morphine. (See below, paragraphs 28 to 32). Thus, most of the "alkaloid 4" material is excluded anyway by the ether-acetone extraction, but it does not appear that any error is introduced even if the first extraction is made with benzene-isopropanol. The ether-acetone extraction

/may

may be completed more quickly by the use of a much larger proportion of acetone than was originally specified. Although more extraneous material comes over than with 60:40 ether-acetone, it is less than with benzene-isopropanol, and this change in the method seems to be for the better.

21. "Alkaloid 5", which gives a thebaine-like colour reaction with (2 + 1) sulphuric acid, is apparently a phenol and perhaps a diphenol. It has scarcely been found in more than traces in ether or ether-acetone extracts from the lime-water solutions. Most extractions of it are incomplete, and it is associated with the strong development of yellow or brown colours in alkaline solution. It is extracted more readily by chloroform from alkali solution than by chloroform from dichloracetic acid solution. However, if the colour development is indeed due to this alkaloid, it is much more harmful to the morphine determination on that account than by reason of the negligible amount of base that might get into the final morphine residue.

22. "Alkaloid 6" is perhaps to be identified with lanthopine. It is a very weak base which is readily extracted by chloroform from dichloracetic acid solution, and is also extractable by chloroform from alkali solution.

23. Either "alkaloid 7" or "alkaloid 3" is presumably codamine; although the green colour given by nitric acid -- which is supposed to identify codamine -- is very transient, in fact a glaucine-type reaction, as actually observed with residues of "alkaloid 7" or "alkaloid 3". "Alkaloid 7" closely accompanies "alkaloid 2" (laudanine), and in spite of discrepancies in the description it probably is codamine, which is an isomer of laudanine. At any rate "alkaloid 7" is extractable by chloroform either from dichloracetic acid solution or from alkali solution.

Other Possible Alkaloids

24. Thebenine is a phenolic base which does not occur naturally, but is formed from thebaine when the latter is boiled in a strongly acid solution (e.g., 10 per cent HCl). Treatment with hot acid solution, sufficiently drastic to convert the thebaine, has been used in some proposed methods of opium assay. Thebenine is not extracted from alkaline solution by chloroform, but it is extracted by chloroform from dichloracetic acid solution. After the latter shakeout in an experiment, ammonifying the aqueous solution and extracting with chloroform-isolutanol yielded

/only

only a slight residue with no titration. Thus, even if thebenine were present at the start, it does not appear that it could produce any error in the morphine titration by the United Nations method. Due to the formation of coloured products from the readily oxidizable thebenine it is inadvisable to use any method of extracting the opium which will convert the thebaine. Of course, the extraction of the opium with lime-water, as in the present United Nations method, does not produce any thebenine.

25. Orthodiphenol-oxymorphine - This name is used here for the oxidation product of morphine which is an orthodiphenol; it is formed in various oxidations of morphine instead of pseudomorphine. Some of this substance was tried in the morphine method. After addition of ammonium chloride to the lime solution, ether-acetone extracted more than half the quantity (about 26 mg. out of an original 40 mg.) in a few hours. It was not extracted readily by chloroform from dichloracetic acid; 10 shakeouts extracted only about 8 mg. If present in any substantial amount, some would probably get into the final morphine residue. However, after taking care to remove residual traces of morphine, an attempt to titrate about 13 mg. showed no titration value whatever. This substance has never been identified in opium, nor has any evidence of it been found in the morphine determinations made by the United Nations Secretariat.

26. Pseudomorphine, an oxidation product of morphine, is almost certainly not a natural alkaloid of the poppy plant. In the factory separations by which it has been obtained from opium it was probably formed by the chemical processing; but the possibility of small amounts being present in some opiums can hardly be excluded. Pseudomorphine was tested in the United Nations method under discussion. It was soluble in lime-water, and though it was thrown out of solution when ammonium chloride was added, very little (only some 3 mg. out of 40 mg.) was carried over by ether-acetone, in several hours extraction. Pseudomorphine, like morphine, is not extracted by chloroform from dichloracetic acid solution. The base is not readily soluble in organic solvents, but with the conditions the same as for morphine, more or less is extracted (with chloroform-isobutanol 21 mg. were recovered out of 50 mg.). Furthermore, although a much weaker base than morphine, it does yield a titration value. Since pseudomorphine is probably not substantially present in opium, and is scarcely extracted by ether-acetone, about

/the only

the only way it could be present in the residue dissolved by dichloracetic acid would be as a result of formation from morphine in the ether-acetone receiving flask, or in the course of evaporation of the solvent solution. In such circumstances a little would get into the final morphine residue, but the net effect would be negative, due to destruction of morphine. Any morphine converted into pseudomorphine in the original lime-water solution would almost all be lost. Thus, if any pseudomorphine should be found in the course of the determination, it would most probably indicate a real loss of morphine, rather than an apparent gain. No indication of pseudomorphine has been noted in the extractions made by the Secretariat, but some damage to the morphine molecule, not resulting in recognizable pseudomorphine, does occur (see below, paragraphs 28 to 32).

27. Neomorphine is an isomer of morphine, having the same relation to it that neopine does to codeine. It has never been shown to occur in opium, but since neopine seems to be a natural opium alkaloid, it is reasonably probable that a little neomorphine occurs naturally, too. Some known neomorphine was kindly furnished to the United Nations Secretariat for experiments by Dr. Lyndon F. Small, National Institutes of Health, Bethesda, Maryland, U.S.A. Although neomorphine is very much more soluble in water than morphine, it is extractable under the same conditions. During extraction, however, and while the free base is in solution, the molecule is very easily damaged, presumably by oxidation, so that identifying colour and crystal tests are injured or even destroyed. Some doubtful residues obtained from opium extracts were studied, and were concluded to be damaged morphine, not damaged neomorphine. No definite evidence for the presence of neomorphine in opium was found in these studies, and it seems unlikely that there can be any significant error in the morphine titration because of the presence of neomorphine. However, the scientific question of its possible presence in opium in very small proportion is still an open one.

28. Damaged Morphine - When ether containing peroxides was inadvertently used for several ether-acetone extractions, it became extremely difficult to extract the damaged morphine from ammoniacal solution in the next-to-last stage of the method; so difficult, in fact, that it was almost impossible to complete the extractions. Moreover, the crystal tests for morphine in the late extracts were spoiled by the damage to the molecule. The colour tests were less affected; at most the response to Marquis' reagent was slightly delayed, the colour with Fröhde's

/reagent

reagent more blue, and the Mecke's reaction much weaker. The damaged morphine still yielded a titration. The damage to the morphine molecule was presumably due to oxidation, but it was not found possible to detect pseudomorphine.

29. It is imperative that pure ether be used for alkaloidal extractions. The ether should be tested by shaking a small portion with some acidified KI solution. If a brownish-yellow colour appears promptly, the ether needs to be purified, which can be done by shaking with $NaHSO_3$ solution and then washing with water. Numerous warnings have been previously given that the purity of ether used for alkaloidal extractions must be verified or an anti-oxidant routinely used. The article by Charles E. Morgan, chemist of the New York Racing Commission, "Extraction of Minute Amounts of Morphine", (Industrial and Engineering Chemistry, Analytical Edition, 9, 383-384 (1937)), although relating only to micro quantities of morphine, may be particularly mentioned. Mr. Morgan has informed the United Nations Secretariat chemists that sodium sulphite solution has been found a more useful antioxidant than pyrogallol.

30. When pure ether is used in the United Nations method, there is usually not enough damaged morphine to cause any concern. However, with certain samples some difficulty in completing the final extraction is noted, particularly if tests are made with Marquis' and Fröhdé's reagents. (See the discussion of Stage IV of the original method, paragraph 35 of ST/SOA/SER.K/1). In such cases it is possible that the total amount of damaged morphine, though very small in comparison with the amount formed by impure ether, may still be over 0.1 per cent of the opium, and the question arises whether it is all formed in the course of the ether-acetone extraction, or whether some damaged morphine may be present in the opium, before analysis is begun.

31. When some of the morphine is damaged during the analytical process (but not so much as to make subsequent extraction impossible), the effect is only to lower the final titration value, due to difficulty in completing the final extraction. On the other hand, if some damaged morphine already exists in the opium before analysis, it ought not to be included (even partially) in the assay; but with normal raw opium such a positive error must be quite small, and perhaps not even appreciable.

/32. Further

32. Further experiments will be made on methods of preventing any damage to the morphine molecule during the analytical process. These will include: (1) Use of antioxidants; (2) Shortening the time during which the morphine is present as the free base, or undergoing extraction, or in alkaline solution; (3) Substitution or use for comparison purposes of other solvent combinations not containing such a sensitive solvent as ether, e.g., benzene-acetone or benzene-isopropanol. When everything feasible has been done to ensure that no damage is done to the morphine during the analytical process, it will be possible to decide whether any "damaged morphine" already exists in some types of opium before analysis.

Conclusion

33. The principle on which the United Nations method for assaying the morphine content of opium is based is the thoroughly sound one of isolating, weighing, and titrating all the morphine and nothing but morphine from a given quantity of opium or a suitable aliquot of the lime-water extract. In this paper the elimination of the other naturally occurring alkaloids of opium -- both major and minor, non-phenolic and phenolic -- has been accounted for, to the limits of our present knowledge and availability of the known alkaloids. In a subsequent paper, various possible means for improving the United Nations method will be presented.

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The Colorimetric Determination of "Porphyroxine-Meconidine".

Communication on Experiments to Find a Relation Between "Red Lovibond Units" and Absorption Density Value of the Red "Porphyr" Solution

By Dr. L. Fuchs and Mr. W. Ullrich,
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In continuation of the international programme of scientific research undertaken pursuant to resolutions 159 II C (VII) and 246 F (IX) adopted by the Economic and Social Council on 3 August 1948 and 6 July 1949 respectively, the Secretary-General is presenting the following paper contributed by collaborating scientists of Austria. The paper establishes a correlation between photometer readings and the red Lovibond units used by the Secretariat, so that those who do not have the Lovibond may be able to interpret colorimetric readings for "porphyroxine-meconidine" in determining the origin of opium.

The Colorimetric Determination of "Porphyoxine-Meconidine"

Communication on Experiments to Find a Relation Between "Red Lovibond Units" and Absorption Intensity Values of the Red "Porphyr" Solution

By Dr. L. Fuchs and Mr. W. Ullrich

1. Purpose of the paper: the documents E/CN.7/195, ST/SOA/SER.K/4 and all others issued by the United Nations about "porphyroxine-meconidine" give the figures for intensity of the red colour in "red Lovibond units". As most interested laboratories in Europe do not possess a Lovibond Tintometer it will not be possible for them to make direct use of the research work on "porphyroxine-meconidine" done by the United Nations Secretariat. Moreover, we see no advantage in measuring with undefined, conventional units, where the reproducible extinction values for a certain wavelength could be used. For these reasons we have tried to work out a key for converting the "red Lovibond units" into extinction values and vice-versa.

2. Our determinations were carried out according to E/CN.7/195 on 27 of the samples listed in ST/SOA/SER.K/4. The absorption intensity of the red colour was measured with the Pulfrich Stufenphotometer of Zeiss using the green spectral filter S 53 (maximum of permeability 528 mμ, effective centre 530 mμ), and some comparative readings were made with a Beckman Photometer. The filter was chosen after the absorption-curve had been determined by means of the Beckman Spectrophotometer, and a suitable maximum had been found at the wavelength of 520 mμ. To find a relation not only in regard to values obtained by instruments with the above mentioned filter, but also to values obtained by photoelectric instruments with strict monochromatic light, we determined the absorption intensities of ten samples with the Beckman Spectrophotometer at the maximum of 520 mμ. The results of the Beckman readings proved to be higher, and the reason is clear from the absorption curve. It shows a rather steep decline after the 520 mμ maximum, thus explaining the lower values at the effective centre of 530 mμ of the S 53 filter.

3. As no calibration-curve is available to give any idea of the absolute quantity of "porphyroxine-meconidine", we think it the more important to make possible a comparison of the colour intensities.

/4. Suitable

4. Suitable concentrations of the red solution for photometer readings were obtained when the volume was made up to 100 ml. In practice, the colour was developed, brought to 10 ml with 5 volume-per cent acetic acid, and 1 ml of this solution was again diluted to 10 ml. This dilution (1 per cent w/v referred to opium) proved to be suitable for measurement in the 1 cm cell of the Beckman and the Zeiss instruments. In the case of samples with extremely high values the 1/2 cm cell of the Zeiss Stufenphotometer was used. Only extremely low values require the first dilution (10 per cent w/v referred to opium) for the determination, and in our experiments this was necessary in one case only (opium Korea No. 1, B, Lov. value 2.5). For this reason all absorption intensities are given in our Table in terms of the coloured solution of 100 ml from 1 g opium and in the 1 cm cell; in the following referred to as $E^{1}_{1\ cm}$ per cent w/v.

5. The fact that in nearly all cases the 1 g: 100 ml dilution could be used and that for the colorimetric determination 10 ml of the red solution is quite sufficient, gave us the idea of developing a semi-micro method with 0.15 g opium, on which we are now working.

6. It seemed very important to us to take a sample of average composition for the determination. This can hardly be done, if the fresh opium is used. Most of the samples are inhomogeneous, covered with varying envelopes, and the moisture-content in the outer parts of a loaf is lower than inside. The opium samples of the United Nations were therefore prepared as follows: the envelope (poppy leaves, paper, Rumex fruits, etc.) was removed, the lump reduced to small pieces and dried in a vacuum-exsiccator for five days. The loss of weight ranged from 2.11 to 5.88 per cent, being an average of 3.99 per cent. After this the opium could easily be powdered and was stored in glasses sealed with paraffin. For the determination a quantity of this dried and powdered opium was used which was equal to 1.5000 g fresh opium.

7. In the attached table we present the results of our studies. The first column gives the absorption intensities obtained with the Zeiss Stufenphotometer under the conditions described above, the samples being listed in the order of increasing extinction-values. The correlation between the $E^{1}_{1\ cm}$ per cent w/v values obtained by us (column 1) and the Lovibond units (column 3) considered for all samples, gave an average of 0.0251 $E^{1}_{1\ cm}$ per cent w/v per 1 red Lovibond unit.

/One sample

One sample ("Yugoslavia No. 2") was not considered for this average, as no loaf was stated on the label. Ten out of the 26 samples showed a deviation of more than ± 10 per cent from this average value. These are marked with an asterisk in column 4. To be sure that these large deviations were not due to errors in the determination, a second determination of these ten samples was carried out and it showed no higher deviation than ± 1.5 per cent from the middle value. After that it was clear that the differences in the results were caused by the nature of the opium (other loaves, varying moisture, different samples, etc.). For this reason, the mentioned ten samples were left out of consideration in setting up the final correlation between the Lovibond units and the absorption intensities. The final average result from the remaining 16 samples was:

$E^{1}_{1\ cm}$ per cent w/v per 1 red Lovibond unit = 0.0253

Pulfrich Stufenphotometer Zeiss, filter S 53.

To find the correlation to Beckman Photometer values, ten samples of an average of 0.0253 $E^{1}_{1\ cm}$ per cent w/v (Zeiss Photometer) per Lovibond unit were measured with the Beckman Photometer. The values at the maximum of 520 mμ were found to be 13.13 per cent higher than the Stufenphotometer values on the average. We can therefore establish the correlation:

$E^{1}_{1\ cm}$ per cent w/v per 1 red Lovibond Unit = 0.0286

Beckman Spectrophotometer, model DU, 520 mμ.

9. Conclusion: in the hope of establishing a uniformly accurate correlation between Lovibond units and absorption intensities we were disappointed, as our United Nations samples showed deviations from the values given in ST/SOA/SER.K/4 up to ± 28 per cent. But as we left the samples with very high deviations out of consideration and took the average of the remaining samples, the figures given above can well be taken, in general, for the correlation between red Lovibond units and absorption intensities. We hope that our results will be a help to institutions which want to carry out examinations concerning the origin of opium and do not possess a Lovibond Tintometer. To establish an accurate "calibration" of the different colorimetric equipments it would be best to distribute a well mixed, dried, and powered opium with a well-defined Lovibond value as a standard to all interested laboratories.

/TABLE

TABLE

Opium	$E^{1}_{1\,cm}$ per cent Stufenphotometer, filter S 53	2nd determination	Lovibond Units	$E^{1}_{1\,cm}$ per cent per 1 red Lovibond Unit	
Korea No. 1	0.067		2.5	0.0268	
Turkey No. 4, Çal (B)	0.219		8.0	0.0273	
" No. 6, Nallihan (B)	0.236		10.0	0.0236	
" No. 7, Merzifon (B)	0.255	0.257	9.0	0.0283	*)
Greece (d)	0.258	0.264	12.8	0.0202	*)
Korea No. 2	0.288		11.6	0.0248	
Turkey No. 3, Çorum (B)	0.313	0.308	10.0	0.0313	*)
Indochina, Laokay (k)	0.312	0.321	13.8	0.0226	*)
Yugoslavia No. 1	0.333		12.4	0.0268	
China (B)	0.336		13.5	0.0249	
Turkey No. 8, Burdur (B)	0.370	0.373	18.0	0.0205	*)
" No. 15, Exportation	0.376	0.380	11.6	0.0324	*)
" No. 13, Isparta	0.387		16.0	0.0242	
" No. 11, Akhsehir (B)	0.438	0.439	22.4	0.0196	*)
" No. 2, Afyon	0.452		18.0	0.0250	
Indochina Xieng Khouang (B)	0.458		19.2	0.0239	
Turkey No. 5, Usak (B)	0.460		18.4	0.0250	
Indochina Luang Prabang (B)	0.474		20.0	0.0237	
Turkey No. 1, Aydin (C)	0.484	0.495	17.2	0.0281	*)
Yugoslavia No. 2 (C)†	0.534		21.8†	0.0245†	
Turkey No. 14, Malatya (C)	0.586		23.2	0.0253	
" No. 9, Kula (B)	0.602		23.9	0.0252	
India No. 3, Excise	0.749	0.755	27.0	0.0277	*)
Turkey No. 10, Sandikli (B)	0.852		32.6	0.0261	
India No. 2, Malwa	0.894		35.8	0.0250	
" No. 1, Banares	0.920		34.6	0.0266	
Turkey No. 12, Zile (C)	1.168	1.176	64.0	0.0182	*)

Figures of this table obtained by Pulfrich Photometer Zeiss, filter S 53.
*) marks the samples with a deviation of more than ± 10 per cent from the average value.

† As mentioned in the text, this sample was not used in computing the equivalent, because the loaf sent was not marked on the label. The loaf designation (c) and the Lovibond value have been supplied by the Secretariat from their records.

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THE ASSAY, CHARACTERISTICS, COMPOSITION, AND ORIGIN OF OPIUM

No. 11

Assay of Raw Opium for Morphine

A study of the Method of Professor Erich Knaffl-Lenz

Laboratories of the S.E.M.P.A. Company, Paris.

In continuation of the international programme of scientific research undertaken pursuant to resolutions 159 II C (VII) and 246 F (IX), adopted by the Economic and Social Council on 3 August 1948 and 6 July 1949 respectively, the Secretary-General is presenting the following paper prepared by chemists of the S.E.M.P.A. Company, in which the method of assay given in ST/SOA/SER.K/3 is further considered and modified.

52-42862

ASSAY OF RAW OPIUM FOR MORPHINE

A Study of the Method of Professor Erich Knaffl-Lenz

(ST/SOA/SER.K/3, 1 August 1951).

Work done in the laboratories of the Company S.E.M.P.A., Paris.

1. The Knaffl-Lenz method has been tried experimentally on a Turkish opium. It gave the results described by the author: constant and higher by 0.3 in the percentage of morphine than the Harrison assay.

2. Because the first filtration of the opium extracts appeared to be difficult through a crucible-filter, the following method was adopted: filtration through a Buchner funnel, diameter 5-6 cm., with two layers of filter paper; for returning the opium into the flask, use of a spatula and a brush without lifting off the paper; filtration of the aqueous solution saturated with sodium sulphate through a layer of 10 g of fine washed sand.

3. Another inconvenience is the formation of emulsion and the precipitation of sodium sulphate during the extraction of morphine. It is well to prepare the solution of sodium sulphate at least two days ahead of time and to keep it at a temperature of 15° to 20° C. In spite of these precautions, sometimes an annoying emulsion still forms.

4. It was found necessary to agitate the alkali solution twice with benzene to eliminate the secondary alkaloids completely.

5. The analysis requires much longer than the 2-3 hours stated. To this must be added sufficient time to dry the opium, pulverize it and dry it again. The exact determination of the loss of weight is important. In fact, we needed a day and a half to apply this method.

6. The morphine obtained by evaporation of the solvent is still very coloured. The fact that the result is generally higher than the Harrison assay does not prove that it is exact.

7. It seemed good in the light of previous experiences to try to modify this method while keeping its best points, in order to obtain a more pure morphine prior to titration.

Principle of the Modified Method

8. We came back to extraction with lime water, done at room temperature, which already provides for the separation of resins, organic acids, colouring substances, and also part of the secondary alkaloids. It is possible to perform this extraction totally, without dividing it up, by using a method suggested by M. MASCRE for the assay of opium. (Ann. Pharm. Fr. 3, n° 3-4 - 1945, p. 111).

9. Though this extraction requires a little more time it is easily applied and in fact less tedious, because the filtrations and the extractions using solvents are easier to perform. The prior drying of opium can be avoided which saves time and eliminates a possible source of error.

10. The excess of lime is afterwards eliminated by precipitation as carbonate in the presence of an excess of sodium hydroxide; the remaining codeine is extracted with benzene.

11. After concentration of this solution brought to pH_4, the greater part of the tarry material is precipitated by sodium sulphate and the morphine extracted, after addition of sodium carbonate, by the solvents recommended by Kraffl-Lenz. (It has been verified that in presence of ammonia, the extraction is incomplete.)

12. After evaporation, one can purify the morphine and crystallize it before the titration.

13. The purity of the morphine obtained would be better if one could find a more selective solvent, and esp :ially one not miscible with water.

14. Thus modified, this method can be applied within a day and a half. It gave constant results close to those of Harrison and of the International method.

15. It constitutes a new milestone towards the establishment of a basic method for the assay of morphine in raw opium, which will help in the future to find faster physical-chemical methods. This method can be applied without modification to the assay of morphine in poppy plant material or more exactly in the dry extract obtained from aqueous-alcoholic extracts of dried poppy plant material.

Description of the Modified Method

16. For the assay, crush 3 grammes of opium in a small mortar with 10 cc of water heated to 60°. When the opium is disintegrated as completely as possible,

add 1 g of calcium hydroxide and work to a very fine paste, then add 10 cc of cold water and stir for about 10 minutes. Filter through a crucible filter of fritted glass (No. 3 G 3) into a suction flask of 250 cc, so that the foam is not troublesome. Crush in the mortar 0.5 g of calcium hydroxide with 5 cc of water and transfer it to the crucible. Bring the caked residue into suspension again by means of a stirring rod; this operation must be done very carefully. To the fine paste obtained add 10 cc of water which has been used for rinsing the mortar, and again stir for several minutes before filtering by means of suction.

17. Wash the dry residue 3 times in the same way with 10 cc of cold water. Add 5 cc of water for mixing and another 5 cc of water before stirring and sucking dry. (These filtrations take 3/4 of an hour, but need little attention.)

18. Add hydrochloric acid to the alkaline solution to slight acidity (about 0.9 cc of acid, sp. gravity 1.19). Heat the solution to about 60° and add 5 cc of 5 N sodium hydroxide and 3 cc of a 10 per cent anhydrous sodium carbonate. Stir it and cool rapidly while letting the precipitate settle.

19. Filter through a crucible filter, first decanting the clear solution, and then carrying along the precipitate; and wash with the minimum amount of water.

20. The solution is diluted to 105 cc in a graduated flask. Transfer it without rinsing into a dry separatory funnel and agitate with 80 cc of hot benzene. Filter most of the water layer, cool it and measure from it exactly 70 cc corresponding to 2 grammes of opium.

21. Pour this solution into a small pyrex evaporating dish and add hydrochloric acid drop by drop to pH_4. Then concentrate on the steam bath ot 15 - 20 cc and again adjust to pH_4 if necessary. Then add 2 grammes of anhydrous sodium sulfate, which is rapidly dissolved by stirring with a glass rod. After cooling, filter through a very small pledget of cotton. The rinsing is done with a saturated solution of sodium sulphate (which has been left for 2 days at 15° - 20°) to which is added 0.5 cc 0.1 N HCl for each 100 cc. The dish and the funnel are washed with 2 cc of this solution. Then transfer the piece of cotton to the dish, and wash the funnel with 5 cc of wash solution. Then heat it for several minutes on a steam bath, cool, and filter it through a second pledget of cotton. Repeat this washing once more.

22. To the solution add 5 cc of a 10 per cent solution of anhydrous sodium carbonate. and extract by shaking with 80 cc of a mixture of chloroform and ethanol in the proportion of 3 to 2 (sp. gravity = 1.220). Then use successively 50, 20, and 20 cc of a mixture of chloroform - ethanol in the proportion of 3 to 1. (Specific gravity = 1.300). Shaking can be carried out without taking precautions; if a little emulsion is formed it can be broken easily by placing the separatory in a horizontal position for some seconds or by heating its bulb slightly, with the stopcock open, on a steam bath. It is necessary to wait before separating until the lower layer of solvents has become clear.

23. The entire mixed solvent solution is dried by shaking twice with anhydrous magnesium sulphate. Filter through cotton and wash the salt quickly with the solvent mixture. Finally evaporate the solution on a steam bath in an Erlenmeyer flask, and complete the evaporation by means of a vacuum pump.

24. The dried residue may be titrated directly - or purified by crystallization. (The more impure the morphine is with gummy substances, the greater is the risk of losing morphine in this purification and then the result will be too low.) Dissolve the residue in 2 cc of a mixture of methanol-benzene in a proportion of 1 to 3, then add 10 cc of water saturated with morphine, chilled with iced water for half an hour and filter off the morphine on a crucible-filter, washing with a few cc of cold benzene, then with ether. Dissolve the morphine remaining in the Erlenmeyer flask in 2 portions of 10 cc each of N/10 sulphuric acid and transfer the solution to the crucible in order to dissolve all the morphine; it is necessary to crush this morphine with a glass rod in the bottom of the crucible. Then wash through with water and titrate the excess of acid with N/10 sodium hydroxide in the presence of methyl red until the solution turns yellow.

25. One cc of N/10 acid corresponds to 0.0285 g of anhydrous morphine. The result obtained corresponds to 2 g of opium.

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THE ASSAY, CHARACTERISTICS, COMPOSITION, AND ORIGIN OF OPIUM

No. 12

Determination of other titratable alkaloids in the morphine of opium assays

In continuation of the international programme of scientific research undertaken pursuant to resolutions 159 II C (VII) and 246 F (IX), adopted by the Economic and Social Council on 3 August 1948 and 6 July 1949 respectively, the Secretary-General is presenting the following paper prepared by the United Nations Secretariat chemists. The assay of opium is subject to both positive and negative errors, which vary with the method of assay. The present paper deals particularly with the determination of the positive error due to the presence of other titratable alkaloids contaminating the assay morphine in both precipitation and extraction assays.

52-10825

THE DETERMINATION OF OTHER TITRATABLE ALKALOIDS IN THE MORPHINE OF OPIUM ASSAYS

Discussion

1. Progress in the opium assay through the years has in general resulted in an increase in the amount of morphine proved to be present in normal opium of various origins. We cannot, however, merely compare two different assay methods by their results, and conclude that the one yielding generally higher results is the better and more accurate. If the problem were as simple as that, it would only be necessary to increase the positive correction which is applied in several pharmacopoeial methods of assay, until there could be no question of its being high enough. We cannot even say that the precipitation method or the extraction method yielding the highest uncorrected results is the best of its class, until we have studied its possibilities of positive as well as negative errors.

2. While the ideal method will be free from substantial errors, either positive or negative, one less ideal in this respect may be shorter, faster, often useful, and generally satisfactory, provided that a true correction can be applied. A true correction can be ascertained if a balance-sheet can be drawn up experimentally for each type of opium, of positive and negative errors, and it can be applied in practice if the net correction is nearly enough the same for all types of opium, or if it is constant for particular types, and the type of opium is known at the time of the analysis. Such a balance-sheet begins to be possible with the use of the dichloracetic-acid separation introduced by the Secretariat.

3. Most methods of assay prior to the Secretariat's method explained in ST/SOA/SER.K/1, have included quite appreciable positive errors due to the presence of other titratable alkaloids contaminating the assay morphine. This applies both to precipitation methods and to extraction methods. The exceptions are perhaps, a few methods that have combined separation by extraction with precipitation of crystalline morphine from a relatively pure solution.

4. Examples of the last-mentioned methods are the method of Eder and Wäckerlin of 1937 (Quarterly Journal of Pharmacy and Pharmacology, 10, 690-730, 1937), the method of Eder and Wäckerlin of 1940 (Pharmaceutica Acta Helvetiae 15, 227, 1940), the method of Dowzard, Thomas, and Russo (Journal of the American

Pharmaceutical Association 26, 615-618, 1937), and the method of Baggesgaard-Rassmussen and Jespersen (Dansk Tidsskrift for Farmaci, 1937, 278-289).

5. Such methods doubtless are accurate, since the precipitated morphine is relatively pure and the correction for morphine remaining in the mother-liquor is relatively certain. However, they are complex and include most of the extraction methods. and yet give up the objective of determining the morphine without any correction. In short they are compromises, originating in recognition of the defects and undetermined errors of the usual precipitation methods, and inability to get the desired purity of the final morphine by extraction alone.

6. The simplest modification of the usual precipitation procedure by the use of an organic solvent was proposed by Annett and Singh in 1918, and consisted merely of separating the non-phenolic alkaloids from the line-water extract of the opium, by means of toluene, before precipitation. The morphine itself was not extracted. According to Annett and Singh, codeine causes the retention of additional morphine in the mother-liquor, and its removal thus diminishes negative as well as positive errors. The percentage of morphine by the British Pharmacopoeia process was increased by the toluene modification in assaying Indian opium. This article, "The effect of codeine in hindering the precipitation of morphine by ammonia from a solution of its lime compound" (Analyst 43, 205-213, 1918), has never had the attention it appears to deserve.

7. The straight precipitation methods, which are official in the Pharmacopoeias, include a positive error due to co-precipitation of other titratable alkaloids with the morphine, and often one due to titration of small amounts of lime compounds precipitated with the morphine. The latter is particularly discussed in "De Morphinebepaling in Opium" by M.A.G. Smeets (Thesis, Rijks-Universiteit, Utrecht, Netherlands).

8. These positive errors in precipitation methods are quite appreciable, even though they are greatly over-balanced by the chief negative error, the amount of morphine left in solution. Often a large positive correction is applied to compensate: but the correction, when one is used, is uniform, although the error certainly varies with the type of opium, if not even from sample to sample.

9. The determination of this principal error of precipitation methods has special difficulties, due to the accumulation of impurities with the relatively

small amount of morphine remaining unprecipitated. This problem will be studied later.

10. The other alkaloids in precipitated morphine have sometimes been determined, in the past, by extraction from alkali solution, usually with benzene. This was assumed to take out only non-phenolic alkaloids, and left the question unanswered, whether any of the minor phenolic alkaloids might be co-precipitated. As a matter of fact, such determinations of other alkaloids in the assay morphine are now found to be very nearly correct, at least for methods using lime-water extraction of the opium, because there is scarcely anything but codeine to be determined. However, the employment of chloroform extraction from dichloracetic acid solution rather than solvent extraction from alkali, for determining other alkaloids in precipitated morphine, gives assurance that contamination by the minor phenolic alkaloids is not being overlooked.

11. Determination of the impurities in the assay morphine have also been made in the past by determining the percentage of methoxyl present. The objection to this is that the methoxyl may be partly due to alkaloids that are not titratable, or even to non-alkaloidal substances.

12. Extraction methods were previously based on a separation by means of alkali solution and an organic solvent. It was generally taken for granted that the minor phenolic alkaloids accompanied the morphine to the final titration, but this possible error was commonly neglected by proponents of extraction methods, and over-emphasized - as an unknown quantity - by proponents of precipitation methods, or methods using precipitation after extraction.

13. Actually, as the Secretariat chemists have already shown, several of the minor phenolic alkaloids are separated from morphine by chloroform from alkali solution, though usually not (or at least not readily) by carbon tetrachloride or ether. However, an appreciable amount of phenolic alkaloid, about 0.3 per cent of the opium weight when calculated by the morphine factor, remains with the morphine even when chloroform is used. It consists chiefly of "alkaloid 3". When only a non-chloroformic immiscible solvent is used for this separation the positive error in the morphine determination will normally be greater.

Method

(For precipitation methods and for extraction methods other than the the Secretariat's dichloracetic-acid separation.)

14. After the titration of the assay morphine, if methyl alcohol was used to dissolve the morphine, evaporate the neutral solution just enough to drive it off. Then pour the solution into a separatory funnel, add a very little dilute H_2SO_4 in excess of neutrality, and shake out the indicator with two or three portions of CCl_4.

15. To the solution add 30 cc $CHCl_3$ and then $NaHCO_3$ to alkalinity to litmus, with a small excess; and shake. Transfer the $CHCl_3$ with shaking through the following solutions: in separatory #2 - 10 cc water plus 0.4 cc dichloracetic acid; #3 - 7.5 cc water plus 0.1 cc dichloracetic acid; #4 - 5 cc water (some dichloracetic acid is carried into this water by the $CHCl_3$); #5 - 10 cc water plus 10 drops concentrated NH_4OH; #6 - 10 cc wash water. In transferring the $CHCl_3$ from separatory #1 to #2, filter it through a pledget of cotton in a short-stemmed funnel. Finally filter the $CHCl_3$ from separatory #6 through a pledget of cotton into a weighed dish.

16. Shake out five times more with portions of $CHCl_3$ of 25 to 20 cc, then discard the original solution. Be sure that the solution in separatory #5 remains ammoniacal, and is not made acid by dichloracetic acid carried along.

17. If at any time it is considered necessary to check on the non-removal of morphine, take a tiny to small portion of the extract as it is transferred from separatory #5 to separatory #6. Evaporate the portion in a small white porcelain crucible, cool, and test with Frŏhde's reagent. No morphine reaction should be obtained. A different reaction from that of morphine is to be expected at first. When the Frŏhde test has become absent or negligible, test small portions also with Marquis' and Mecke's reagents (Codeine).

18. In the usual shake-out, test the sixth portion of $CHCl_3$ with Frŏhde's, Marquis', and Mecke's reagents, as just described. In shaking out the assay from an extraction process, if the tests indicate that some opium alkaloid is still being extracted, continue the shakeout from the dichloracetic-acid solutions with fresh portions of $CHCl_3$ (20 - 15 cc), until any other alkaloid is completely extracted, as long as morphine does not appear.

19. In the shake-out of the assay from a precipitation method, the tests on the sixth portion of $CHCl_3$ will probably show nothing but codeine still being extracted. In this case make the acid solutions (separatories 2, 3 and 4) alkaline with NaOH solution adding an excess. Two or three more portions of $CHCl_3$ will then remove the remaining codeine.

20. Finally, the $CHCl_3$ extracts in the weighed dish are evaporated. In the case of "other alkaloids" from a precipitation assay, some CCl_4 may then be added and evaporated off, whereupon, with a little continued heating on the steam bath, the residue will probably crystallize, as it is chiefly codeine. Cool the dish with the residue in a desiccator, and weigh. Then dissolve the residue in a little methanol, add methyl red indicator, and titrate with n/50 or n/100 H_2SO_4, with the addition of boiled distilled water. The residue of course is not morphine but may be calculated in terms of morphine, to express the result as an error of the morphine determination.

Method of ST/SOA/SER.K/1

21. The assay morphine of the method used by the Secretariat (ST/SOA/SER.K/1) has already undergone the dichloracetic-acid separation in the course of the assay. "Other alkaloids" can be determined in it in a similar way to the method just given, but substituting alkali solutions for the dichloracetic-acid solutions. Three alkali solutions are advisable, and the chloroform should be allowed to settle well after shaking and preferably be filtered from one separatory to the next, as traces of morphine are much more easily carried along by chloroform from alkali than is generally realized. The "other alkaloids" thus extractable yield a titration of only about 0.5 cc n/50 acid (equivalent to 0.07 per cent of the opium weight by the morphine calculation), when the dichloracetic-acid separation has been thoroughly carried out.

Some Results and Conclusions

22. The Secretariat chemists made a number of determinations of the titration of non-morphine alkaloid extractable by chloroform from dichloracetic-acid but not by chloroform from alkali. The results, beginning with the old League of Nations samples, (calculated as for morphine as percentage of the opium weight) were:

I.	Benares	0.33 per cent
III.	Iran	0.34, 0.35 per cent
IV.	Turkey	0.35 per cent
V.	Yugoslavia	0.30 per cent
Singapore sample (Far Eastern type of opium)		
		0.36, 0.32 per cent
	Japan	0.33 per cent

23. These determinations were discontinued as it seemed they could have no bearing on the origin problem, because the percentage was so nearly the same for different types of opium.

24. A determination of the other alkaloids in the assay morphine from a trial of the Buchbinder procedure showed 0.43 per cent of the opium weight as the positive error.

25. A few determinations were tried on the assay morphine of the Knaffl-Lenz method (ST/SOA/SER.K/3), but as the assays themselves were not altogether satisfactory, due to inexperience with the method, the results were somewhat inconclusive. In one case the "other alkaloids" found amounted to 0.93 per cent of the opium weight. This doubtless included some codeine, for, as pointed out in ST/SOA/SER.K/11 from the laboratories of S.E.M.P.A., a single extraction with benzene is not enough to eliminate even the non-phenolic alkaloids completely.

26. It is clear that any extraction method using merely the alkali separation, and which claims to have no negative error, needs a negative correction (to balance the positive error) of atleast 0.3 per cent of the opium weight.

27. This does not condemn such extraction methods in comparison to precipitation methods, for if the error is known, it is just as easy to make a negative correction as a positive one in the calculation; and perhaps considerably more accurate, having regard to the nature of the errors. However, such methods should be scrutinized very carefully to be sure that they really do not include any negative errors, before it is concluded that their results are necessarily too high.

28. The "other alkaloids" were determined by dichloracetic-acid separation on four assays by the method of the British Pharmacopoeia, two on an Indian opium and two on a Far Eastern opium with morphine contents of about 12 and 13.7 per cent.

The results in terms of the opium weight (calculated as for morphine) were 0.47 0.45, 0.55, and 0.42 per cent.

29. This material extracted from the B. P. assay morphine gave the analytical reactions of codeine and crystallized readily, as codeine. Scarcely more than traces of other alkaloids were present.

30. The results correspond closely to one stated by Rakshit (Analyst 56, 711-713, 1931). From 200 grammes of the assay morphine (B.P. 1914), he obtained 11.3 grammes of other alkaloids by extraction from alkali with benzene, i.e., 5.65 per cent by weight of the assay morphine. From 5 grammes of this material he obtained 3.6 grammes of crystalline codeine; there was no proof but that nearly all the rest may have been codeine, too. The total percentage in terms of anhydrous morphine calculated from titration would therefore be about 5.0 per cent of the morphine value.

31. The Secretariat results also correspond closely to the contamination found for U.S.P. assay by Rosin and Williams (Journal of the American Pharmaceutical Association 24, 1053-1062, 1931). By extraction with chloroform from alkali they obtained values (calculated as morphine as a percentage of the opium weight) of 0.45, 0.34, 0.53, 0.45, 0.39, 0.35 per cent; average 0.42 per cent.

32. The methoxyl determinations of Baggesgaard-Rasmussen, Jackerott, and Jespersen on the assay morphine of the International methods also correspond (Pharmaceutica Acta Helvetiae, Nr. 11, 1936). Calculated as codeine they represent 3.9 to 7.0 per cent of the morphine, representing an average, calculated as morphine error, of 4.85 per cent of the morphine value.

33. However, the contamination may often be greater. Smeets obtained higher methoxyl values than those cited. Recently the Saint Paul Laboratory of the United States Internal Revenue Bureau found U.S.P. assay morphine to contain 0.75 per cent of the opium weight (calculated as morphine), of non-morphine alkaloids, as determined by extraction from dichloracetic-acid. The Saint Paul Laboratory had obtained higher U.S.P. results on the particular sample than several of the other United States laboratories, which may possibly have a bearing on the high percentage of "other alkaloids".

34. It may be concluded that the "positive error" due to coprecipitation of other alkaloids is at least 0.4 to 0.5 per cent of the opium weight, and comes almost

entirely from codeine. In extraction methods using alkali separation it is nearly or quite as great, 0.3 to 0.4 per cent of the opium weight at a minimum, and due to phenolic alkaloids, especially "alkaloid 3". In the Secretariat's method, however, (ST/SOA/SER.K/1), the known "positive error" is reduced to less than 0.1 per cent of the opium weight. In the pharmacopoeial precipitation methods the "positive error" is overshadowed by the "negative error" of morphine remaining in solution, and this must be further studied.

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THE ASSAY, CHARACTERISTICS, COMPOSITION AND ORIGIN OF OPIUM

No. 12

Determination of other titratable alkaloids in the morphine of opium assays

Corrigendum 1

Page 3

The second sentence of paragraph 5 should begin,

"However, they are complex and include most of the extraction of extraction methods"

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THE ASSAY, CHARACTERISTICS, COMPOSITION AND ORIGIN OF OPIUM

No.13

Exhaustive extraction of the opium with lime-water in the assay procedure

In continuation of the international programme of scientific research on opium, undertaken pursuant to resolution 159 II C (VII) and 246 F (IX), adopted by the Economic and Social Council on 3 August 1948 and 6 July 1949 respectively, the Secretary-General presents the following paper prepared by chemists of the United Nations Secretariat. The question of exhaustive extraction in comparison to a single extraction of the opium with lime-water is an important one for most methods of opium assay. It is taken up in this paper with particular reference to the method explained in ST/SOA/SER.K/1, the assay method used by the United Nations Secretariat.

53-00668

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EXHAUSTIVE EXTRACTION OF THE OPIUM WITH LIME-WATER IN THE ASSAY PROCEDURE

Objectives of the paper

1. The objectives of this paper are the following:
   (a) to show that exhaustive extraction of the opium may be used in the first stage of the Secretariat's method of assay, ensuring solution of the morphine and avoiding the taking of an aliquot;
   (b) to explain the advantages and disadvantages of exhaustive extraction of the opium;
   (c) to show the conclusions that may be drawn from the experiments of the Secretariat with exhaustive extraction.

Discussion

2. At the time the method of opium assay given in ST/SOA/SER.K/1 was worked out two questions relative to Stage I were temporarily postponed: the questions of complete solution of the morphine of the opium, and the exactness of the aliquot. A review of the assay problem will make clear the reasons for this postponement and the role of exhaustive extraction in Stage I.

3. The pharmacopoeial methods of opium assay, and some of the other current methods, use a lime-water solution of the opium extractives as the basis of the morphine determination. That, however, is about as far as agreement goes. Certainly, the results are widely different.

4. For example, Smeets (Die Morphinebepaling in Opium, Thesis, Rijks Universiteit, Utrecht, 1949) showed that with six different methods of opium assay, each of which has won considerable acceptance, the results on "Opium IV" ranged from 14.9 to 17.1 per cent, a difference of more than 2 per cent of the opium weight, and the results on "Opium III" ranged from 4.6 to 7.8 per cent, a difference of more than 3 per cent of the opium weight! Lesser but still serious discrepancies are quite common.

5. One major cause of such differences, occurring even with accepted pharmacopoeial precipitation methods, is the large arbitrary "correction" for morphine solubility, amounting to more than 1 per cent of the opium weight, which is added in some of these methods but not in others.

6. Is such a "correction" justified? The question comes down to that of the true amount of morphine in the lime-water solution of opium extractives. Solvent-extraction methods available prior to the United Nations method had never completely settled this, because of doubt as to the amount and effect of the minor phenolic alkaloids, which had been supposed to be inseparable from the morphine by extraction with solvents.

7. The method of ST/SOA/SER.K/1 was offered primarily as a definitive method for determining the amount of morphine in a lime-water extract of opium. This was, in fact, explained in the original discussion of the method (ST/SOA/SER.K/1, paragraph 9). The basis of an exact comparison with the official pharmacopoeial methods, or any other method using a lime-water extract of the opium, was thereby laid. For, as part of the comparison, two identical lime-water solutions can be prepared by the pharmacopoeial (or other) method. One solution can then be processed according to the pharmacopoeial method and the other extracted by the method of ST/SOA/SER.K/1.

8. However, in order to present a complete assay, the Secretariat desired to include a procedure for "Stage I", that is, for obtaining a lime-water solution of opium extractives which would represent some definite quantity of opium.

9. The problem of ensuring that all the morphine is brought into solution, although quite important, is not the cause of the most serious discrepancies between the results by different methods of assay. This is evident from the fact that two of the best-accepted methods, the International method and the method of the British Pharmacopoeia, which are among those yielding the highest results, employ only a single extraction of the opium. On the other hand the method of the United States Pharmacopoeia employs exhaustive extraction, before taking an aliquot, and yet it is among the methods yielding the lowest results.

10. The Secretariat chemists therefore concluded that a single extraction, if carefully made, should be satisfactory. This is especially true, since the morphine of a given quantity of opium is dissolved by a larger volume of solution in the Secretariat method than in pharmacopoeial methods which also use a single lime-water extraction.

11. As regards the aliquot, a great deal of attention has been given to the question of making it theoretically correct on the assumption that the morphine is all in solution; and yet this has even less bearing on the major discrepancies

between results by different methods than the question of completely dissolving the morphine. The questions of the proper solubility correction and its variation with different types of opium, in precipitation methods, and of the minor phenolic alkaloids in the older solvent-extraction methods, far overshadow the question of a theoretically perfect aliquot, which can have only a very minor effect in determinations on the usual raw opium.

12. The method of the United States Pharmacopoeia and the International method both go to great pains to make the aliquot theoretically correct, or nearly so (assuming complete solution of the morphine); but they do not agree at all in results, nor is there any real evidence that the over-all accuracy of either is superior to that of the British Pharmacopoeia method, which uses simply an approximate volume aliquot.

13. The Secretariat chemists concluded, therefore, that a simple volume aliquot, modelled on that of the British Pharmacopoeia, would be most satisfactory for the time being. Extra trouble to determine an exact aliquot is not justified as long as any increased accuracy thus gained is completely swallowed up by larger uncertainties.

14. The United Nations Secretariat method, like the methods of the British Pharmacopoeia and the United States Pharmacopoeia, determines the morphine in the opium "as is", and a calculation of the morphine content of anhydrous opium, or any reliable comparison with the results of others on opiums of varying moisture content, requires a moisture determination. The determinations made by the Secretariat on opiums of different origins have been made on "air-dry" samples. This further limited any possible variations of the aliquot, and made approximate comparisons feasible without the moisture determinations. There was, however, no intention of setting up "air-dry" opium as an exact standard. The moisture content as determined by the International method is about 5 to 7 per cent in the "air-dry" samples in this laboratory, and of course any fairly precise comparison with the results of others still requires moisture determinations.

Exhaustive Extraction in Stage I

15. Eder and Wäckerlin showed that a very considerable error may be made in the morphine determination - ranging from 0.77 to 1.37 per cent of the opium weight in their experiments - by assuming that all the morphine had been brought into solution by a single treatment with lime-water. ("The determination of Morphine in Opium", Quarterly Journal of Pharmacy and Pharmacology, 10, 680-730, 1937).

16. The Secretariat experiments have been more favorable to the single extraction than this, as will be shown. Nevertheless an incomplete single extraction must rank as one of the major possibilities of error and perhaps the chief reason that inexperienced analysts so often get too low results, regardless of the type of method used in later stages. At the best, there is still an uncertainty about a "one-shot" extraction that only an exhaustive extraction can avoid.

17. The ideal exhaustive method would provide some simple test for completion of the extraction; however, this does not seem to be practicable at present, in using lime-water extraction. The best alternative is to use a procedure that can be depended on to exhaust all the morphine from the opium, regardless of its percentage or the type of opium.

18. Eder and Wäckerlin employed exhaustive extraction; and to ensure complete solution of the morphine, they used 52 cc lime-water in seven portions for 1 gramme of opium, or 250 cc in seven portions for 5 grammes of opium. The experiments of the Secretariat indicate a very similar ration of volume as necessary to exhaust the opium. Some other methods have prescribed fewer treatments and a smaller total volume in proportion to the weight of opium taken, but such procedures may leave a doubt whether the morphine is completely extracted.

19. Thus the most important advantage of exhaustive extraction is ensuring complete solution of the morphine. This is especially valuable to the inexperienced analyst, even though the single extraction may be satisfactory in the hands of those skilled in opium analysis.

20. In the International method moisture and extract determinations are made in addition to the morphine precipitation, in order to calculate a theoretically exact aliquot. But uncertainty remains, because only a single extraction is made.

21. Exhaustive extraction is more certain, and yet avoids the necessity of moisture and extract determinations, which may not be needed otherwise. It likewise avoids any possible inaccuracy of an approximate volume aliquot. This is of value in extending the method to forms of opium for which the specified volume aliquot might be inappropriate, e.g., smoking opium with a high content of moisture.

22. Therefore, although it is quite possible to use the single lime-water extraction and an approximate volume aliquot and still obtain a good analysis on the usual raw opium, it is better to use exhaustive extraction if it does not cost too much in time and trouble.

23. In short, exhaustive extraction has every advantage over the single extraction except that it entails more work and yields a much greater quantity of solution for a given weight of opium. In the United Nations Secretariat method the greater volume of solution means the use for the first solvent extraction, of at least two continuous extractors, or better three, instead of one - if the extractors are of the size previously used, and if the extract is still to represent 4 grammes of opium (see E/CN.7/195 for a diagram of the solvent extraction apparatus used). The solvent extracts can be combined for evaporation, and thereafter the procedure can be the same as for the single extraction with lime-water.

24. In the method of the United States Pharmacopoeia an exhaustive extraction of the opium is made with plain water, and the extract is then evaporated down to a comparatively small volume and converted to a lime-water solution, from which an aliquot is taken. This is necessary in order to have only a small volume for precipitation; but this method also involves considerable time and trouble, and possibly some elements of uncertainty. It might be used to reduce the volume if only a single continuous extractor is available.

25. In view of the preceding "Discussion", paragraphs 2 to 14, it should now be shown that the exhaustive extraction is worth the extra trouble: that the method of the Secretariat, as a whole, is such that the gain in certainty in Stage I is not thrown away or completely overshadowed by more serious uncertainties later on. Otherwise the shorter and faster procedure of a simple volume aliquot would still be preferable for Stage I.

26. The method of the United Nations Secretariat eliminates any question of the amount of morphine normally remaining in solution, and any question of codeine seriously affecting the result; questions which affect the pharmacopoeial precipitation methods. It also eliminates at least the known error, due to minor phenolic alkaloids, of previous extraction methods. Studies are being pursued to determine whether any positive error, due to the inclusion of some unknown titratable substance, with the morphine, is still possible. If so, it has not been established, and cannot be large. It seems, therefore, that the greatest remaining uncertainty is, not the aliquot as such, but the complete solution of the morphine in the lime-water. Therefore the exhaustive extraction is advisable for a rigorous determination in spite of the added time and trouble.

27. Some studies are still under way to determine the best combination of solvents for the extraction of Stage II. In the following method, benzene-isopropanol is directed for the first extractor and benzene-ether-acetone for the second. However, either combintation could be used for both extractions. Benzene-isopropanol is advantageous for speed, while benzene-ether-acetone yields a remarkably "clean" extraction.

Method

28. Weigh out 4.000 gm of the opium into a large mortar. Add 15 cc water and 2 gm Ca $(OH)_2$, grind up and rub to a smooth paste, then add 50 cc more water and continue rubbing and stirring during about 30 minutes. In the meantime put 1 gm more Ca $(OH)_2$ in a flask with about 200 cc water and shake occasionally. Use this lime-water for subsequent extractions.

29. Filter the opium solution with suction, preferably using a Buchner-type sintered-glass filter. (If faucet suction is used, use a trap to be sure there is no back-flow of water into the filtrate.) Suck the residue as dry as possible. Pour this filtrate into a 100 cc graduate.

30. Scrape the opium back into the mortar with a glass rod, and add enough lime-water (about 25-30 cc) to make up the difference between the volume of filtrate already in the graduate and 70 to 75 cc. Rework for about 15 minutes, then filter with suction as before, and pour the filtrate into the graduate.

31. Charge a continuous extractor with the filtrate, NH_4Cl, and 200 cc of benzene-isopropanol (150 : 50). Use an amount of NH_4Cl as follows: for 70 cc lime-water solution, 2 gm; for 75 cc, 2.15 gm; for 80 cc, 2.3 gm NH_4Cl. In charging the extractor, to prevent the aqueous liquid from running through the porous plate, pour in the aqueous solution and part of the solvent solution at the same time. Then connect the apparatus and pour the remaining solvent in through the condenser. Begin extraction at once. Continue until the extract from a small portion of the returning solvent, evaporated in a small porcelain crucible, no longer shows an indication of morphine with Fröhde's reagent (0.5 gm sodium or ammonium molybdate in 100 cc concentrated H_2SO_4).

32. With the suction cut off, rinse any opium marc remaining in the mortar onto the filter with about 20 cc lime-water and work up the opium marc thoroughly on the filter; then apply the suction. Pour the filtrate into the graduate. Rework

the opium marc on the filter, with the suction cut off, three times more, with about 20 cc lime-water each time, collecting a total of about 75 cc lime-water extract.

33. Charge a second continuous extraction apparatus with the filtrate sufficient NH_4Cl (paragraph 31), and 100 cc of ether-acetone (60 : 40). As soon as the extraction apparatus is charged with the opium solution, connect it and pour any remaining ether-acetone in through the condenser. Also pour in through the condenser 75 cc of benzene-acetone (45 : 30). Stand the extraction flask in a beaker containing a little water on a hot plate, and extract as usual. Test for completion of the extraction with Maquis or Frõhde's reagent.

34. Return the opium marc to the mortar and rework with 25 cc lime-water. Filter. Rework twice more on the filter, with the suction turned off, with about 25 cc water each time, to collect a total of about 75 cc more lime-water extract. Put in a third continuous extractor with NH_4Cl and 150 cc of ether-acetone (90 : 60). Extract as usual, testing for completion with Frõhde's reagent.

35. The solvent extracts from the first two extractors, or from all three, can be combined during evaporation to yield a single residue, which is then processed for morphine as usual. In the Secretariat experiments the extract from the third extractor was processed separately, in attempting to work out the method so as to require only two extractors. It may still be advisable to process it separately for a few times, while an analyst is inexperienced, as a check on the technique.

36. In Stage III, if the residue from the third extractor is processed separately, it is sufficient to use two acid solutions. Besides 25 cc chloroform, 0.3 cc dichloracetic acid and 10 cc water are used in transferring the residue to the first separatory. The second separatory contains 5 cc water, with no dichloracetic acid added directly (the chloroform carries some along). Extract the other alkaloids with additional portions of 25-15 cc $CHCl_3$. In Stage IV omit the first 50 cc of solvent mixture and begin extracting with 25 cc of chloroform-isolutanol (75 : 25). Test a little of the third or fourth such extract to see if the morphine is already all out. Continue the extraction, if necessary, with 20 : 5 chloroform-isobutanol. In Stage V titrate this residue with n/50 or n/100 acid.

The Single Extraction

37. Weigh out 6.000 gm of opium (preferably, but not necessarily, in the "air-dry" condition); put into a large mortar with 20 cc. water and 3 gm $Ca(OH)_2$, and grind to a uniform paste. Add 70 cc. water and stir thoroughly and very frequently for 30 minutes, carefully rubbing the opium marc to make disintegration as complete as possible. Filter and take 61 cc of the filtrate to represent 4 gm of opium. (This aliquot, which includes an allowance of 1 cc for extractives, is justified by the results given below).

38. In the International method, the lime is added with the first water (as in the preceding paragraph), and the mixture worked up. In the method of the British Pharmacopoeia, the opium is first worked up and thoroughly disintegrated with water alone, before the lime is added. This sometimes makes the process of disintegration more difficult, and the Secretariat chemists have not found convincing evidence that such a precaution is of much help in ensuring complete solution of the morphine. They have found, however, that it is inadvisable to grind the opium and lime together before adding water.

39. In both the British Pharmacopoeia and the International methods, treatment by grinding is followed by rinsing into a flask, and shaking. The Secretariat chemists have not found this necessary, and it seems very doubtful if shaking is any more likely, or even as likely to ensure complete solution of the morphine as continued stirring in the mortar, with rubbing of any opium marc that resists disintegration. On the other hand, the use of a Blendor may sometimes be advantageous, though this has not been rigorously tested. The Blendor produces some foam, but filtration with suction will yield the specified aliquot.

40. Another requisite for successful use of the single extraction is that the lime be full strength. In spite of the fact that the lime is used in considerable excess, this alone is not sufficient, and some analysts prefer to use their own recently slaked lime. However, the commercial $Ca(OH)_2$, which has been well protected from the atmosphere, should be satisfactory. The International method includes a test for the strength of the lime.

Results and observations

41. Although the results by the method of the United Nations Secretariat are generally among the highest, in the case of the old League of Nations samples they were lower than expected from the original analyses of about 1935-1937, on samples III, IV, and V. (Iran, Turkey, and Yugoslavia). There have previously been reports of loss of morphine in powdered opium during storage, and it seems probable that it has occurred in this case. (See, especially, Annett and Singh, "The loss of morphine content of powdered opium on storage", Pharmaceutical Journal 109, 304-305, 1922.) This point will be studied further.

42. Comparisons between the results by exhaustive extraction and satisfactory determinations by single extraction, are as follows:

Sample	Morphine by Single extraction	Exhaustive extraction
L.of N. I. Benares (India)	10.55,10.47	10.66
L.of N. II. Calcutta (India)	11.04	11.18
L.of N. III. Iran	10.57	10.51,10.40
L.of N. IV. Turkey	10.55	10.60
L.of N. V. Yugoslavia	15.29,14.94	15.18
India No.3 - Indore State	9.52	9.56
U.N. 6 - Turkey - Nallihan-B	13.45	13.26
U.N. 49 - Iran - Kerman	11.65,11.81	11.56

43. As far as small discrepancies in these results are concerned, it should be remembered that a difference of 0.1 - 0.2 means a difference of only two or three drops of tenth-normal acid in the titration, at the end of a long and complex separation. The hundredth place is really quite uncertain, but is allowed to stand here to show the comparison of results more clearly.

44. It is only too easy for some accident or error to make a result definitely too low. In the experiments of the Secretariat a low result has occurred a number of times when it has seemed that the deficiency must be due to incomplete solution in the single extraction method. In such cases the results have been low by amounts ranging up to as much as 1.6 per cent of the opium weight. In other words, deficiencies of the order found by Eder and Wäckerlin, or nearly as

great, may occur rather easily when only a single extraction is used, even with the greater amount of lime-water in the method of ST/SOA/SER.K/1 as compared to most precipitation methods. However, the figures show that complete solution can be obtained with the single extraction, on a wide variety of opium types.

45. In the Secretariat's determinations the residue from the third extractor (solvent extraction of Stage II) was processed separately. The amount of morphine found in it was, roughly, about one-half per cent of the total amount; the maximum found was 0.14 per cent of the opium weight, the average was 0.08 per cent of the opium weight. Despite efforts to get all the morphine into the first two extractors, for example by increasing the number of separate treatments of the opium with lime-water to obtain the total volume of about 150 cc., qualitative tests always showed a little morphine obtained from the third extractor, with a titration corresponding to at least 0.03 or 0.04 per cent of the opium weight. In two or three cases a re-extraction was necessary to obtain the titration. It was ascertained that microcrystal identification tests for morphine, as well as the characteristic colour tests, could be obtained on the final residue from the third extractor.

Conclusions

46. A single extraction of opium with lime-water (as in the International and British Pharmacopoeia methods of assay) is capable of giving satisfactory results. However it is difficult to obtain complete solution of the morphine with a single extraction, and almost impossible to be perfectly sure of it.

47. If single extraction is used, the accuracy of a simple volume aliquot (as in the method of the British Pharmacopoeia) is satisfactory for ordinary raw opium. Any uncertainty thus introduced is far overshadowed by others, including the uncertainty of the single extraction itself.

48. Exhaustive extraction is far more certain. It is certainly advisable for a rigorous determination, but is fully justified only when other large uncertainties are eliminated, as in the method of the United Nations Secretariat.

49. Exhaustive extraction requires a minimum of approximately 50 cc lime-water per gramme of opium, in about seven separate treatments. Since complete solution of the morphine is possible with a single extraction, the difficulty of getting it out appears to lie largely in the opium holding the solution like a sponge.

50. In the Secretariat's experiments the very small amount of morphine recovered from the third extractor shows two things: (a) that assuredly all the morphine obtainable by lime-water was exhaustively extracted from the opium; (b) that no titratable substance, capable of staying with the morphine in the Secretariat's method and soluble in line-water but less so than morphine, exists in opium in any considerable quantity - otherwise it would certainly be noted in the final residue from the extracts processed in the third extractor.

51. With exhaustive extraction of the opium in Stage I, the method of the United Nations Secretariat, as far as can be ascertained at present, has all the elements of a rigorous determination of the morphine content of opium.